Executive Summary

On August 30, 1999, the South Florida Water Management District (District) contracted with DB Environmental Laboratories, Inc. (DBEL) to perform a 100-week evaluation of Submerged Aquatic Vegetation/Limerock (SAV/LR) Treatment System technology for reducing phosphorus (P) discharge from Everglades Agricultural Area (EAA) waters. The objectives of this project are to assess the long-term, sustainable performance of this technology, and to develop design and operational criteria for a full-scale SAV/LR system. For this effort, we are performing scientific and engineering work at Stormwater Treatment Area (STA)-1W at several spatial scales: outdoor microcosms and mesocosms, test cells (0.2 ha), Cell 4 (146 ha) and Cell 5 (1,100 ha). This document is a quarterly progress report describing work efforts of DBEL's project team from May - July 2000. Key accomplishments and findings are as follows.

During this quarter, we continued using existing mesocosms at both the North and South Advanced Treatment Technology (NATT and SATT) sites of STA-1W to assess effects of hydraulic loading rates, water type (Post-BMP vs. Post-STA), and system configuration on SAV/LR phosphorus removal performance.

We performed a microcosm study in May to assess the effects of calcium, alkalinity and SAV nutritional status (P starved vs. P enriched) on P removal performance. Soluble reactive P (120 µg/L) spiked into the water column was depleted almost entirely by P starved *Najas guadalupensis* within 5.5 hours, regardless of the hardness and alkalinity regime. By contrast, water column SRP reductions occurred more slowly when the *Najas* plants were P enriched. With P enriched plants, however, SRP was removed from the water at a significantly higher rate under high hardness/alkalinity conditions than under low hardness/alkalinity concentrations. These data demonstrate that hardness and alkalinity characteristics of the Everglades Agricultural Area runoff can have a marked influence on P removal in SAV-dominated STA cells.

During May, we initiated the fluctuating water depth study in NATT mesocosms. In this experiment, water column depths for SAV mesocosms that previously had operated at steady state depths ranging from 0.4 to 1.2 m are being varied in a cyclical fashion. For this study,

under "modified" depth regimes the water levels in the 0.4 m shallow mesocosms were raised to 0.8 m, and the water in the 1.2 m deep mesocosms was reduced to 0.8 m. These "modified" depths were maintained for 5 weeks, after which time the water columns were returned to their original steady state depths. During this quarter, we subjected the mesocosms to two of these depth fluctuation "cycles". To date, the depth fluctuations do not appear to be markedly affecting P removal performance of the SAV communities. The depth fluctuation experiments will be continued through October 2000.

We continued operation of a pulse loading study, using SAV mesocosms that previously had received hydraulic loading rates (HLRs) of 11, 22 and 55 cm/day. Our hydraulic loading schedule for the mesocosms is similar to the "STA-2" hydraulic loading data set in the following respects: our protocol has the same average seasonal flow patterns; the same percentage of "no-flow" weeks; and, the same standard deviation on a seasonal basis. We have scaled (increased) the STA-2 HLRs by factors of 5X, 10X and 25X, with flow provided to the mesocosms in two-week long pulses. These increased flows were selected to match the prior, high HLRs (11 – 55 cm/day) to the SAV mesocosms, which should provide continuity with previous HLR studies.

To date, mesocosms subjected to a pulse-loading regime are providing slightly higher outflow TP concentrations than those that receive a steady state loading. Additionally, under stagnant, "no-flow" conditions, we have observed increases in water column TP levels, as well as phytoplankton blooms. These blooms and elevated TP levels were most pronounced in the mesocosms that received Post-BMP waters at the higher HLRs (i.e., 22 and 55 cm/day).

To compare relative performance of SAV and cattail-dominated wetlands, we continued monitoring of shallow mesocosms containing these respective vegetative communities. The SAV mesocosms continued to outperform cattail mesocosms operated at similar depths (0.4m) and HLRs (10 cm/day), providing mean outflow TP concentrations of 35 and 52 µg/L, respectively, during the quarter.

We continued long-term monitoring of shallow (0.09 m deep) SAV/periphyton/LR raceways that receive Post-STA waters, as well as the deeper (0.4 m) tanks containing SAV cultured on

muck, sand and limerock substrates. In the substrate study, lowest effluent P concentrations were obtained in mesocosms with SAV cultured on a limerock substrate (17 µg/L), and highest effluent levels were observed with SAV cultured on a sand substrate (27 µg/L).

To test the effectiveness of various filter media as a "back-end" particle filtration step for SAV wetlands, we initiated a small-scale filtration study at the SATTS. We fabricated sixteen PVC columns that will be fed outflow water from SAV mesocosms. We will test filter media that are inert, and therefore should only provide particulate filtration, as well as those with chemical characteristics (high calcium or iron content) that should further contribute to soluble P removal. Filter media to be tested include: coarse (3.4 – 6.9 mm), medium (2.0 – 3.4mm) and fine (0.25 – 0.85 mm) quartz pebbles/sand; coarse and medium sized limerock; coarse and medium sized Ca/Mg silicate materials; and a fine, iron-coated quartz sand.

During this quarter, STA-1W Cell 4 inflow waters were collected and passed through a tangential flow filtration device to create discrete size fractions in the following ranges: > 0.40 µm, 0.40 – 0.05 µm, and <0.05 µm. Aliquots of these fractions were transported to the University of Florida Wetlands Biogeochemistry Laboratory, where they are being exposed to a suite of enzymes, as well as varying pH and redox conditions. This experiment will help define the environmental conditions (substrate concentrations, pH and redox) under which recalcitrant P forms (e.g., dissolved organic P) can be converted to labile P (soluble reactive P). These experiments will be repeated next quarter with Cell 4 outflow waters.

Experiments to define the stability of Cell 4 sediments also were initiated this quarter. We collected inflow sediments from this wetland, and subjected them to varying SRP, calcium, pH and redox conditions in our laboratory. We also performed sediment fractionations to determine the relative percentages of labile and recalcitrant sediment P pools, and to assess how the size of these pools change during the laboratory incubations. Our initial results with Cell 4 inflow sediments reveal that sediment P is most stable under oxic, high pH and high [Ca] water column conditions. Additional work with Cell 4 outflow sediments will be performed next quarter.

The 0.2 ha test cells are in a recovery phase, resulting from limerock berm installation in south test cell (STC)-9 and north test cell (NTC)-15, as well as SAV management in the other cells. Presently, we are collecting samples for TP analyses, and the extensive water quality monitoring component of our work plan will be initiated next quarter.

We conducted a literature review to investigate past efforts of modeling P removal in aquatic systems. The purpose of our review was to identify the types of models that have been developed in the past and to assess the utility of various modeling features for our process model development.

In May, we evaluated SAV densities at the 120 monitoring stations that we had established in STA-1W Cell 5 during February 2000. Submerged vegetation, in particular *Najas* and *Ceratophyllum*, are expanding rapidly throughout the wetland, although in general the densities remain fairly low.

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Introduction

On August 30, 1999, the District contracted with DB Environmental Laboratories, Inc. (DBEL) to design, construct, operate, and evaluate a 100-week, multi-scale demonstration of SAV/Lime-rock Treatment System technology for reducing phosphorus (P) discharge from Everglades Agricultural Area (EAA) waters. The objectives of this project are to:

- Design and execute a scientific and engineering research plan for further evaluation of the technical, economic and environmental feasibility of using SAV/LR system for P removal at both the basin and sub-basin scale.

- Obtain samples adequate to conduct a Supplemental Technology Standard of Comparison (STSOC) analysis.

- Provide information and experience needed to design a full-scale SAV/LR system.

This document is a progress report for the third quarter describing work efforts during May – July 2000. This report focuses on methodology and findings from the mesocosm experiments (Task 5), test cell studies (Task 6), Cell 4 sediment stability, hydraulic analysis and performance model (Task 9) and Cell 5 inoculation studies (Task 10).

Task 5. Mesocosm Investigations

During May-July 2000, we continued using existing mesocosms at both the North and South Advanced Treatment Technology Sites of STA-1W to assess effects of hydraulic loading rates, water type (Post-BMP vs. Post-STA), and system configuration on P removal performance. Activities are listed below by experimental subtask.

Effects of Calcium/Alkalinity and Soluble Reactive Phosphorus Concentrations on Phosphorus Coprecipitation (Subtask 5i)

Findings from our Phase I research using Post-BMP waters suggest that P removal in an SAV system is controlled in part by water column hardness and alkalinity. In April, we began establishing and refining the experimental approach to the first two experiments embodied in Subtask 5i. That first experiment entails subjecting previously conditioned, P-enriched and P-starved *Najas* to a constant SRP concentration at high and low levels of calcium and alkalinity over a two-day measurement period to evaluate the relative importance of P uptake by SAV vs. coprecipitation of P in the water column.

We performed screening runs in March and April to obtain data on SRP uptake and release by *Najas*, precision among replicates, source water characterization and the appropriate light and temperature regimes under which to conduct the experiment. Tap water from the City of West Palm Beach was selected as the source water for Experiment 1 because of its consistently low calcium (30 mg/L) and alkalinity (56 mg $CaCO_3$/L) relative to the 71 mg/L Ca and 227 mg $CaCO_3$ alkalinity measured in agricultural drainage water (ADW). We chose to add the desired alkalinity and Ca concentrations to the source water (tap water) rather than attempt to chemically remove them from the Post-BMP ADW. Opting for chemical removal would alter the water chemistry to such a degree that it would invalidate any comparison between low alkalinity/Ca (chemically "softened") and the untreated high alkalinity/Ca waters.

We began the definitive experiment by treating W. Palm Beach tap water for chlorine and SRP removal. This was accomplished by placing water hyacinths (*Eichhornia crassipes*) in the tap water container for six days. After the six-day contact with water hyacinths, the water was then pumped to a contact chamber where inorganic N, potassium, and micronutrient supplements

were added (Table 1). After adding the nutrient amendments, the water volume (~ 20 gallons) was then split in half, and 10 gallons were delivered to each of the two aquaria. Prior to delivery of the replenished water, the *Najas* was removed from each aquarium, and the existing three-day old water disposed. To the aquarium containing the P-enriched *Najas*, we added P at a final concentration of ~ 200 µg/L to the newly added replenishment water. The P-starved and P-enriched *Najas* were then returned to their original aquaria, and allowed to incubate under charcoal sun screen shade cloth in the new growth medium for three days before the entire replenishment process was started anew.

Table 1. Final concentration of nutrients and micronutrients amended to W. Palm Beach tap water during the P-starved and P-enriched conditioning period (35 days) and the final incubation (29.5 hr).

Compound	Nutrient	Final Concentration
KCl	K^+	10.8 mg/L *
NH_4Cl	$NH4^+$	0.5 mg N/L
KNO_3	$NO3^-$	0.5 mg N/L
H_3BO_3	B	10.8 µg/L
$MnSO_4 \cdot H_2O$	Mn	10.8 µg/L
$ZnSO_4 \cdot 7H_2O$	Zn	5.2 µg/L
$CuSO_4 \cdot 5H_2O$	Cu	1.2 µg/L
$(NH4)_6Mo_7O_{24} \cdot 7H_2O$	Mo	0.4 µg/L
$FeCl \cdot 6H_2O$	Fe (in EDTA)	16 µg/L
Na_2EDTA	EDTA	94 µg/L
Cyanocobalimin	B-12	3 µg/L

* 9.4 mg/L from KCl and 1.4 mg/L from KNO_3

Water hyacinths successfully removed both the chlorine and SRP present in the tap water. Using an average six-day exposure period, SRP concentrations were reduced from as high as 118 µg/L to a range of 5 to 20 µg/L; total chlorine residual, which was as high as 3.6 mg/L, was removed to undetectable (<0.1 mg/L) concentrations.

At the end of the 35-day conditioning period, the P-starved *Najas* had nearly the same dry/wet weight ratio as the P-enriched *Najas* (0.063 and 0.067, respectively). However, we found nearly two-fold higher P concentrations in the P-enriched *Najas* (3770 mg/kg) than in the P-starved

Najas (1930 mg/kg). The initial dry/wet weight ratios and P concentrations in the *Najas* inoculum 35 days prior were 0.071 and 2160 mg/kg, respectively.

The tap water, which originally contained 21 mg Ca/L, was spiked with $CaCl_2 \cdot 2H_2O$ to obtain the 85 mg/L concentration for the high Ca treatment. Likewise, an alkalinity-containing salt ($NaHCO_3$) was applied to the tap water to raise the alkalinity level from 52 to 356 mg $CaCO_3$/L.

The following treatments and controls were conducted in triplicate on 2-L volume reconstituted (amended with same concentrations of inorganic N, K, micronutrients as listed in Table 1 plus 125 µg SRP/L) tap water per each 3.785 L incubation vessel:

1. P-enriched *Najas* in low Ca (21 mg/L) and alkalinity (52 mg $CaCO_3$/L) water
2. P-enriched *Najas* in high Ca (85 mg/L) and alkalinity (356 mg $CaCO_3$/L) water
3. P-starved *Najas* in low Ca and alkalinity water
4. P-starved *Najas* in high Ca and alkalinity water
5. Low Ca and alkalinity control water (no *Najas*)
6. High Ca and alkalinity control water (no *Najas*)

Based on our preliminary findings, we introduced 26.4 g fresh wt of either P-enriched or P-starved *Najas* into each incubation vessel containing two liters of growth medium (ratio of 76 mL of medium to 1 g fresh wt plant). *Najas* tissues were briefly rinsed with distilled water (410 mL) before inoculating the culture chambers and after withdrawal at the end of the incubation.

The experimental incubation period began at 1230 on May 25 and ended at 1800 on May 26, 2000. We sampled SRP, total soluble P (TSP), dissolved Ca, total alkalinity, pH, and specific conductance at the beginning and end of the incubation period. In addition, we collected samples for SRP analysis and measured pH after 5.5, 17.5, and 26.5 hours from the onset of the incubation.

The P-starved *Najas* rapidly depleted the initial SRP concentration of 110 or 120 µg/L within the first 5.5 hours of incubation in both the high and low Ca/alk treatments (Figure 1). The SRP

concentrations in the controls (amended medium without *Najas*), on the other hand, remained near their spiked levels of 110 and 120 µg/L. This indicates that in P-starved *Najas*, coprecipitation of Ca and P is irrelevant compared to the nutritional needs of the plant. Because of the high precision among the triplicates, the rate of aqueous SRP decrease was statistically different ($P<0.05$; Mann-Whitney U Test), between the high Ca/alk and low Ca/alk treatments, even though the average slopes were nearly identical (Figure 1).

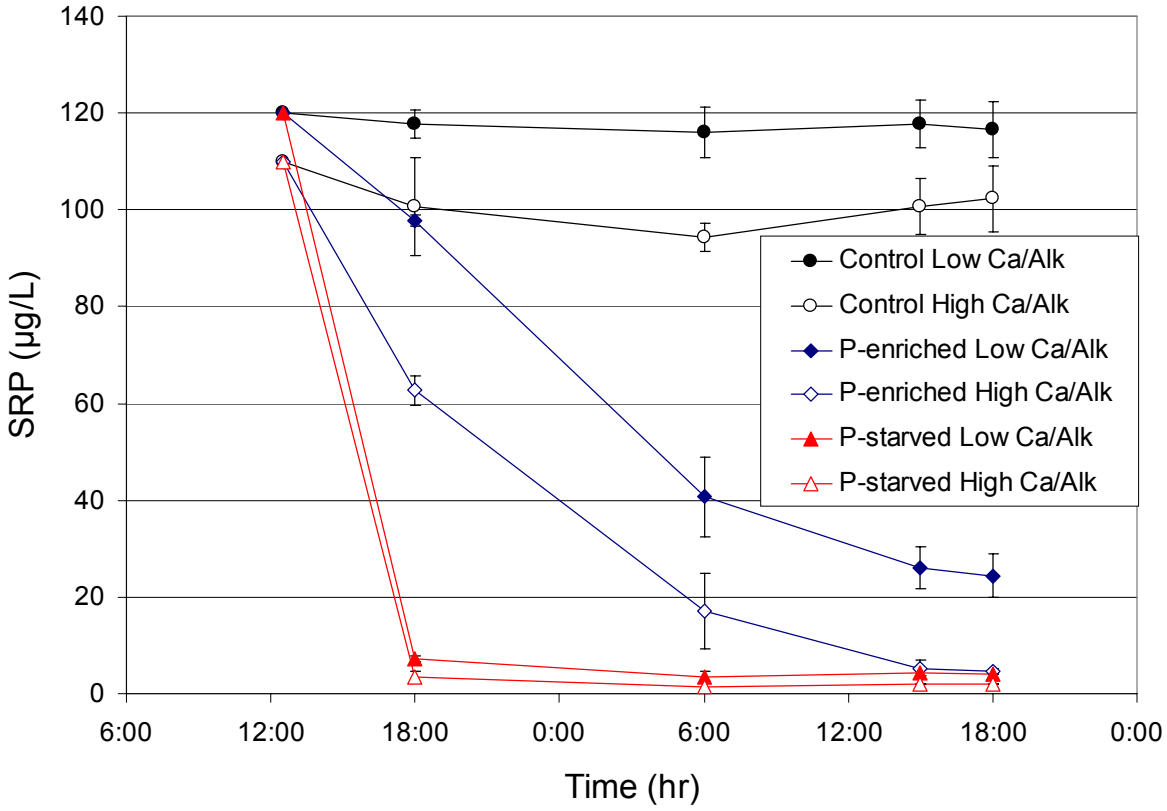

Figure 1. Time course (29.5 hr) for SRP inoculated at 120 µg P/L initial concentration into high and low calcium/alkalinity water containing *Najas* previously cultured under P-enriched and P-starved conditions for 35 days. Controls represent incubation without *Najas*. Each data point represents the mean ± 1 s.d. of 3 replicate flasks. Date of experiment was May 25 – 26, 2000.

However, a different picture emerges when we examine the time course of SRP depletion for the P-enriched *Najas* exposed to high and low Ca/alk treatments (Figure 1). The added Ca and alkalinity constituents in the high Ca/alk treatment caused a two-fold faster decrease in the SRP concentrations within the first 5.5 hours of the incubation than in the low Ca/alk treatment (8.6

µg/L per hour vs. 4.0 µg/L per hour). The slopes of these two treatments during this period are significantly different ($P<0.05$) from each other according to a Mann-Whitney U test. Interestingly, the slopes of the SRP removal for both treatments were nearly equal during the succeeding 12-hour nighttime period. This indicates that the supplemental removal by the P coprecipitating with the calcium carbonate ceased during the night, as expected. The pH values decreased to an early morning low of 7.57 (low Ca/alk) and 8.26 (high Ca/alk) (Figure 2), suggesting a reduced likelihood of $CaCO_3$ saturation.

The reductions in the calcium and alkalinity concentrations were the same in both the P-starved and P-enriched high Ca/alk treatments during the 29.5-hour incubation period (Table 2). This

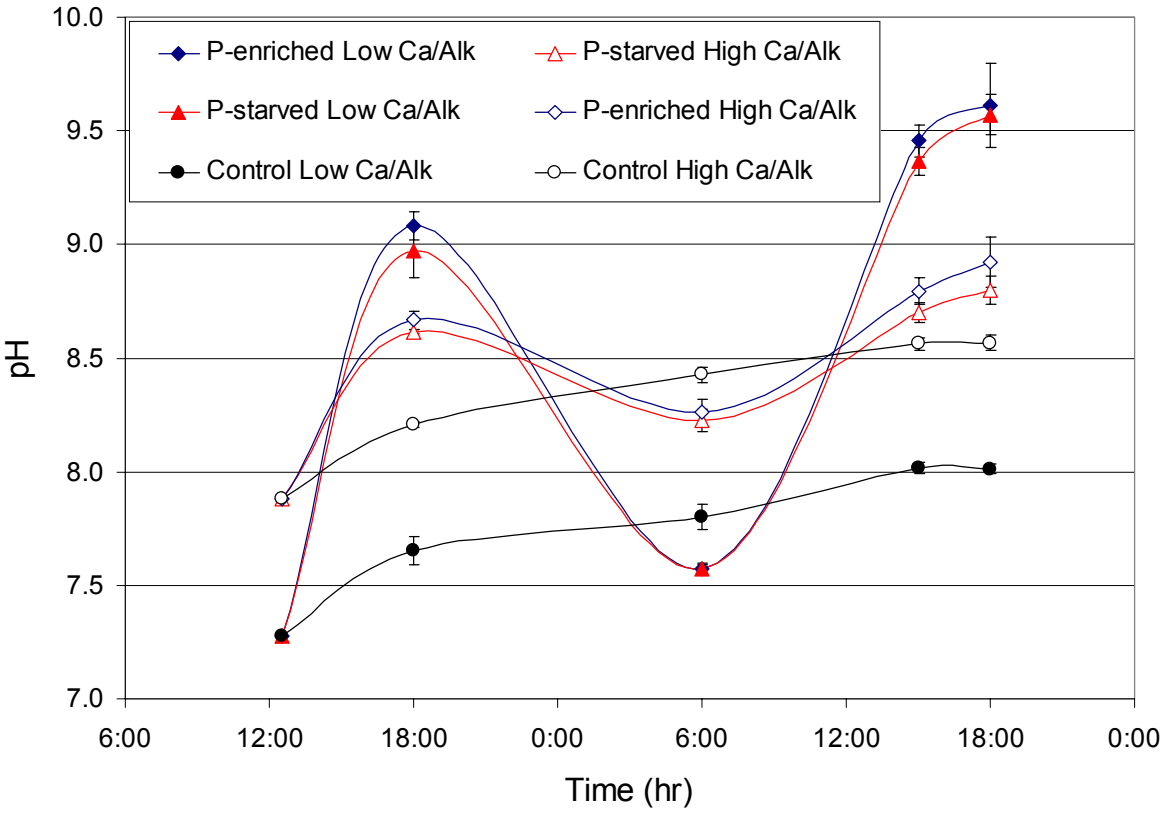

Figure 2. Time course (29.5 hr) for pH after SRP (120 µg P/L) was inoculated into high and low calcium/alkalinity water containing *Najas* previously cultured under P-enriched and P-starved conditions for 35 days. Controls represent incubations without *Najas*. Each data point represents the mean ± 1 s.d. of 3 replicate flasks. Date of experiment was May 25 – 26, 2000.

leads to two significant observations regarding calcium carbonate chemistry. First, a SRP concentration of 120 µg/L does not appear to substantially inhibit the extent of calcium carbonate production. Previous studies (Vaithiyanathan et al. 1997) have suggested that the presence of inorganic P in water column can inhibit $CaCO_3$ precipitation, but this phenomenon was not observed in this study. Second, although SRP was exclusively taken up by the P-starved plant in the high Ca/alk treatment, the extent of calcium carbonate production was the same as for the P-enriched high Ca/alk treatment. This suggests that the precipitation of calcium carbonate is independent of the nutritional status of the SAV as long as sufficiently high pH levels are maintained during the day.

Further insights into the P and Ca chemistry can be found by examining the dry:wet wt ratios and P and Ca concentrations of the P-enriched and P-starved *Najas* at the end of the incubation period. The final dry:wet weight ratios were significantly higher, and the tissue P concentrations significantly lower ($p<0.05$), in the P-enriched high Ca/alk treatment compared to those in the P-enriched low Ca/alk treatment. This was also true for the dry:wet wt ratios in the P-starved treatments, but not for the tissue P concentration. Significant differences ($p<0.05$) were also found between the tissue Ca concentrations of the high and low Ca treatments in both the P-starved and P-enriched plant incubations at the end of the 29.5 hr incubation (Table 2). From 67% (P-starved) to 81% (P-enriched) of the aqueous Ca decreases in the high Ca waters were recovered in the incubated plant tissues. Combining the P and Ca data, it appears that most of the calcium carbonate precipitation is occurring on the surface of the *Najas* plant, which is adding to the dry weight of the plant as well as diluting the concentration of tissue P.

In summary, this is the first direct evidence that we have produced which indicates that the P coprecipitation mechanism does occur within SAV beds. However, the rate and extent are predicated on the nutritional status of the SAV community and on the hardness of the water. These results indicate that the nutritional condition of the plant can be a more important factor than abiotic coprecipitation in removing SRP. In other words, the removal of SRP may depend more on the physiological needs of the plant than on the extent and amount of calcium carbonate precipitation. Some of the calcium carbonate precipitate, regardless of whether it

takes place within a P-enriched or P-starved SAV community, appears to be formed and retained at the leaf surfaces of the plant.

Table 2. Chemical characteristics of water column and plant tissues before and after a 29.5 hr incubation of *Najas* tissues in low- and high-calcium/alkalinity waters. Control values represent flasks without *Najas* inoculum. Plant tissues were pre-conditioned in waters with (P-enriched) or without (P-starved) SRP amendments (See text for details). Final concentrations represent means ± 1 s.d. of triplicate incubation flasks.

	Ca/Alk Treatment	Initial T = 0	Final T = 29.5 hr		
			P-enriched	P-starved	Control
Total Ca (mg/L)	Low Ca/Alk	21	21 ± 1	22 ± 1	23 ± 2
	High Ca/Alk	85	33 ± 8	31 ± 2	85 ± 0
Total Alkalinity (mg $CaCO_3$ /L)	Low Ca/Alk	52	52 ± 0	52 ± 0	56 ± 0
	High Ca/Alk	356	188 ± 21	182 ± 9	358 ± 2
Tissue Ca (wt %)	Low Ca/Alk	16	14 ± 2	16 ± 2	
	High Ca/Alk	16	45 ± 8	38 ± 6	
Tissue P (mg/g)	Low Ca/Alk	3.20 P-starved 6.64 P-enriched	6.24 ± 0.34	3.19 ± 0.29	
	High Ca/Alk	3.20 P-starved 6.64 P-enriched	6.43 ± 0.23	3.60 ± 0.15	

Other factors not explored in this short-term experiment, such as the effects of SRP concentrations on the rate and extent of P coprecipitation, will be pursued in the second experiment where a longer incubation period (3 months) using flow-through systems will be utilized.

Fluctuating Water Depths (Subtask 5ii)

The fluctuating water depth experiment was initiated May 1, with a reduction of the water depth to 0.8 m in duplicate deep (1.2 m) mesocosms at the North Advanced Treatment Technology Site (NATTS). Additionally, the water depth in two shallow (0.4 m) mesocosms was increased to 0.8 m. These depths were maintained for 5 weeks (until June 5), after which time the variable mesocosms were returned to the original depths of 0.4 m and 1.2 m. On July 10, the water levels were again set at 0.8 m in two deep and two shallow tanks (see Figure 3A for the 5-month water depth schedules for all treatments and controls). Three moderate depth tanks were maintained at 0.8 m to serve as control tanks (i.e., static water depth).

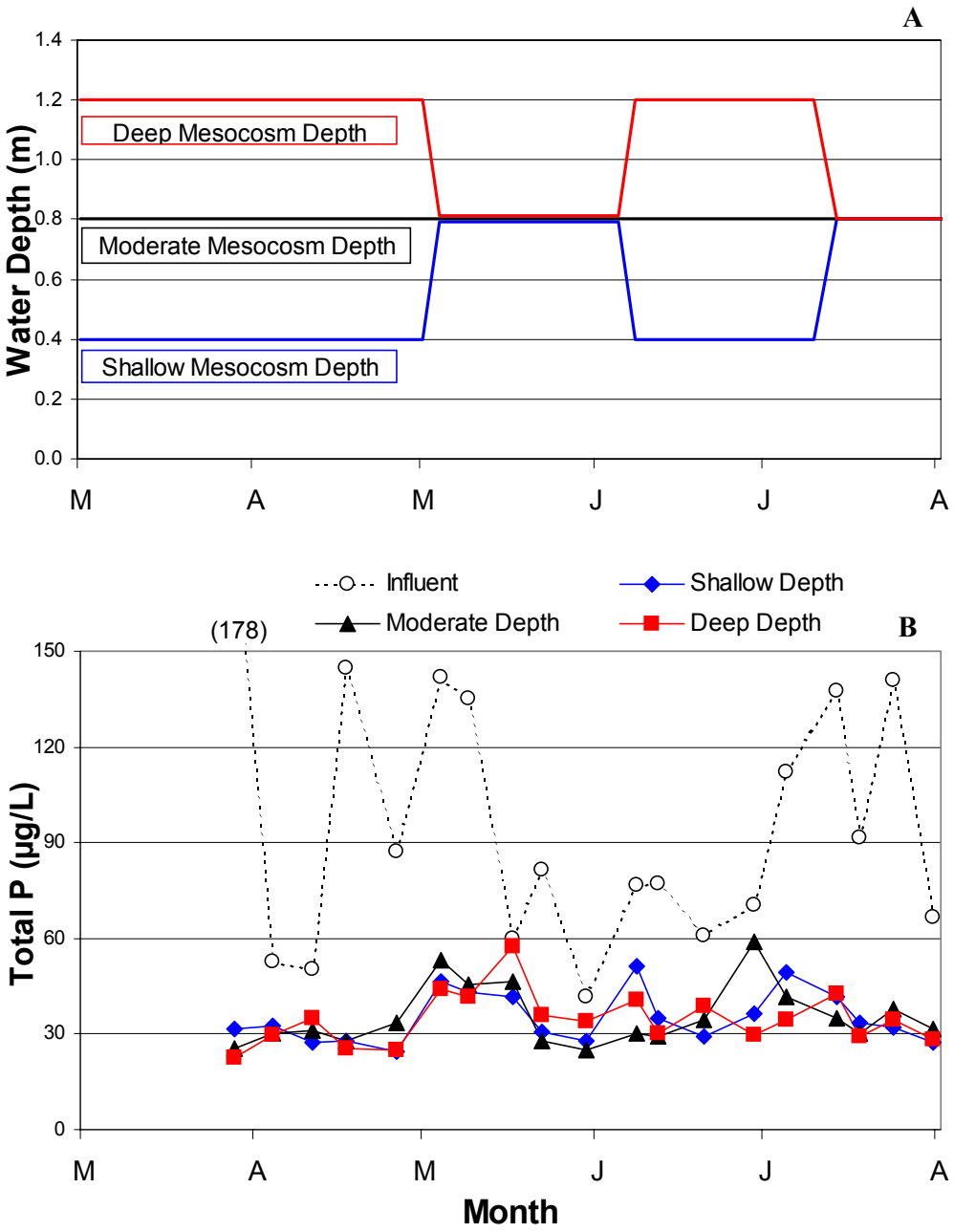

Figure 3. A) Fluctuation depth schedule for deep, moderate (control), and shallow depth mesocosms from March to August 2000. **B)** Mean Total P concentrations in the influent (n = 3) and effluents (n = 2) of deep, moderate (control), and shallow depth mesocosms from March to August 2000.

The process of raising and lowering water depths was performed gradually over a four day period. This cycle of fluctuating depths will be repeated several times during the experiment. One of each of the shallow and deep mesocosms, and all of the three moderate depth (0.8 m) mesocosms, will remain at constant depths for "control" comparisons.

The nine mesocosms in this experiment were sampled for total P and SRP weekly. Background performance was evaluated during the month of April (prior to water depth fluctuation), when the influent TP concentration averaged 104 µg/L. Average effluent concentrations during the background period were similar among the three depths (28-30 µg TP/L). During the first three weeks in May after establishing the new water depths, both the average influent and effluent TP concentrations were higher ($\bar{x}$ = 112 µg/L influent, 42-58 µg/L effluent) for each treatment than during the previous months (Figure 3B). This trend continued through July; average TP concentrations for the May-July quarter in the shallow, moderate, and deep mesocosm effluents were each 37 µg/L.

Mesocosms for Long-Term Monitoring (Subtask 5iv)

Three NATTS mesocosms have been operated since June 1998 at constant hydraulic loading rates (HLR), water depth and hydraulic retention times (HRT). The relative performance of these treatment units has been uniform over the long term (Figure 4). During the May-July quarter, effluent TP concentrations from mesocosms with an HRT of 1.5, 3.5 or 7.0 days averaged 52, 37, and 28 µg/L, respectively. The 1.5-day retention time produced similar effluent concentrations (52 µg TP/L) during the quarter vs. the entire period of record (POR) of 26 months, while the longer HRT mesocosms exhibited higher effluent concentrations over the last three months than during the complete POR (3.5-day HRT for POR: 29 µg TP/L; 7.0-day HRT for POR: 23 µg TP/L).

Pulse-Loading and Drydown-Reflooding (Subtask 5v)

On February 23, 2000, we initiated the 40-week pulse-loading experiment in six mesocosms at the NATTS. Total P concentrations in the effluent region of each mesocosm were monitored twice weekly, while SRP samples were composited on a weekly basis from two grab samples.

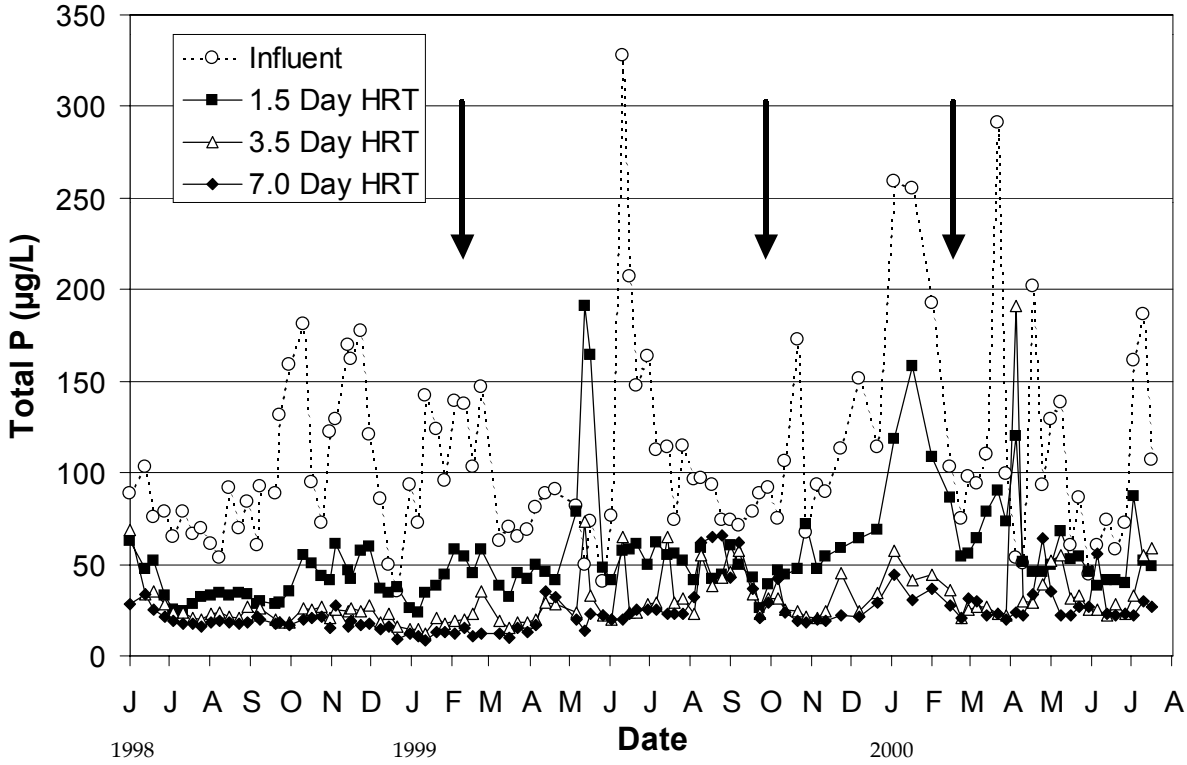

Figure 4. Mean total phosphorus concentrations in the influent and effluents of triplicate mesocosms operated at 1.5, 3.5 and 7.0 day hydraulic retention times (HRT) since June 1, 1998. Data from one mesocosm of each HRT is presented for the period between February 10 and September 29, 1999 (arrows), and after February 23, 2000.

Since start-up in February 2000, there have been four periods of "no flow", where the hydraulic loading to the mesocosms was suspended for two to four weeks. SRP concentrations in the effluents of the pulsed SAV mesocosms did not differ substantially from the effluent concentrations from the control (constant hydraulic-loading) tank within each treatment (Figure 5). Apparently, at least for the highest HLR mesocosms, the inflow SRP concentration is more important in determining effluent SRP concentration than whether the operational mode is pulsed (under the hydraulic loading conditions imposed during the 5-month period) or constant.

The "no flow" periods of April and May appeared to cause increases in total P in the effluent region of the high loading treatment; the same effect was observed (but less pronounced) in the moderate loading treatment, but was absent in the lowest loading treatment (Figure 6). The increased levels of water column TP are likely the result of phytoplankton blooms; we observed

the green alga *Coelastrum* sp. in one replicate of the high loading mesocosms during the "no flow" period on May 23, 2000. Total P concentrations in the mesocosm effluents after the resumption of flow were not significantly different from the mesocosms (Long-Term Monitoring (5iv)) receiving constant loadings (Table 3).

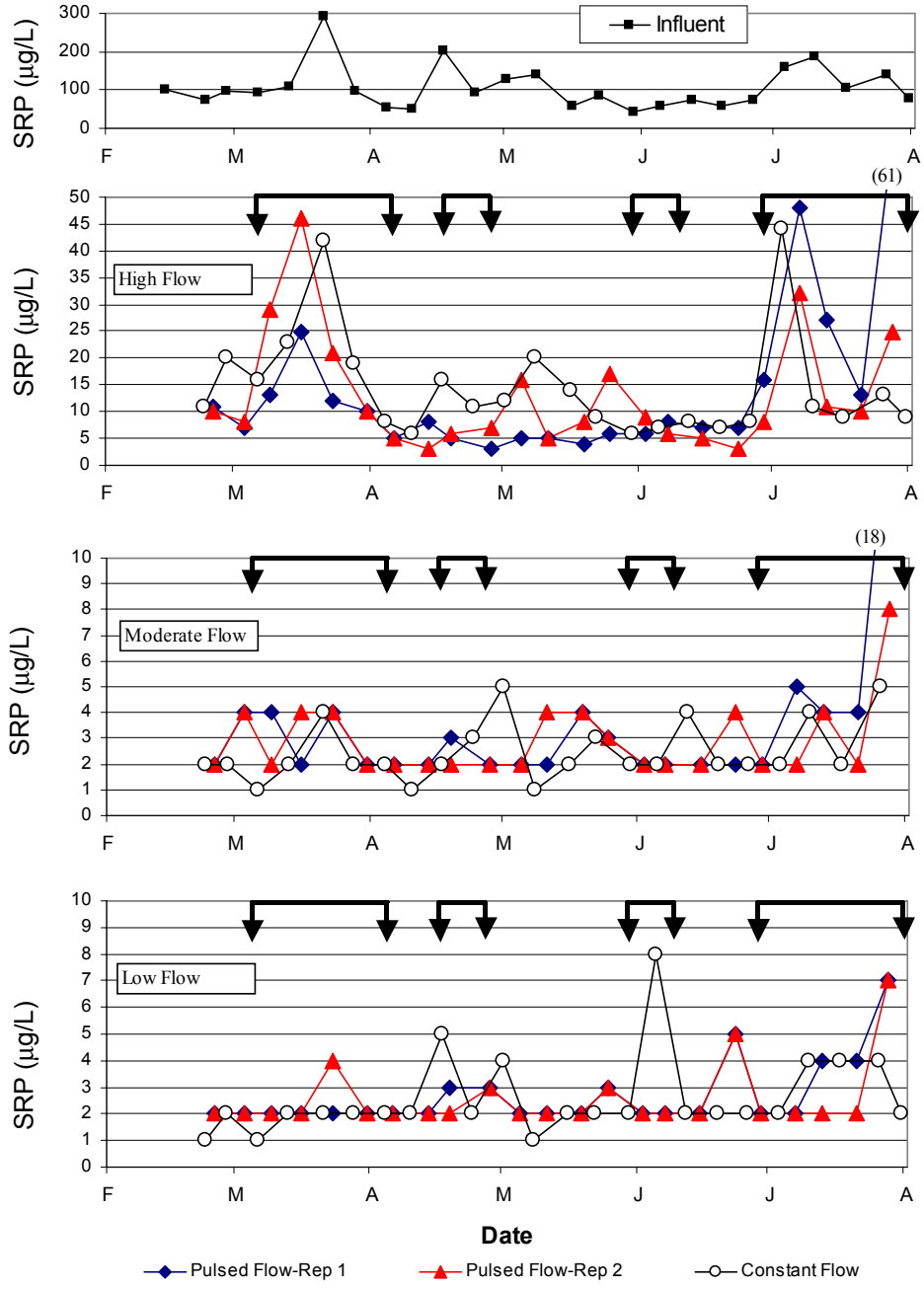

Figure 5. SRP concentration in each of the two pulsed replicate mesocosms and in one constant (unpulsed) flow control mesocosm for high, moderate, and low flow treatments. Arrows bracket periods of hydraulic loading, which are interspersed with no-flow periods. Note the different scales on the y-axes.

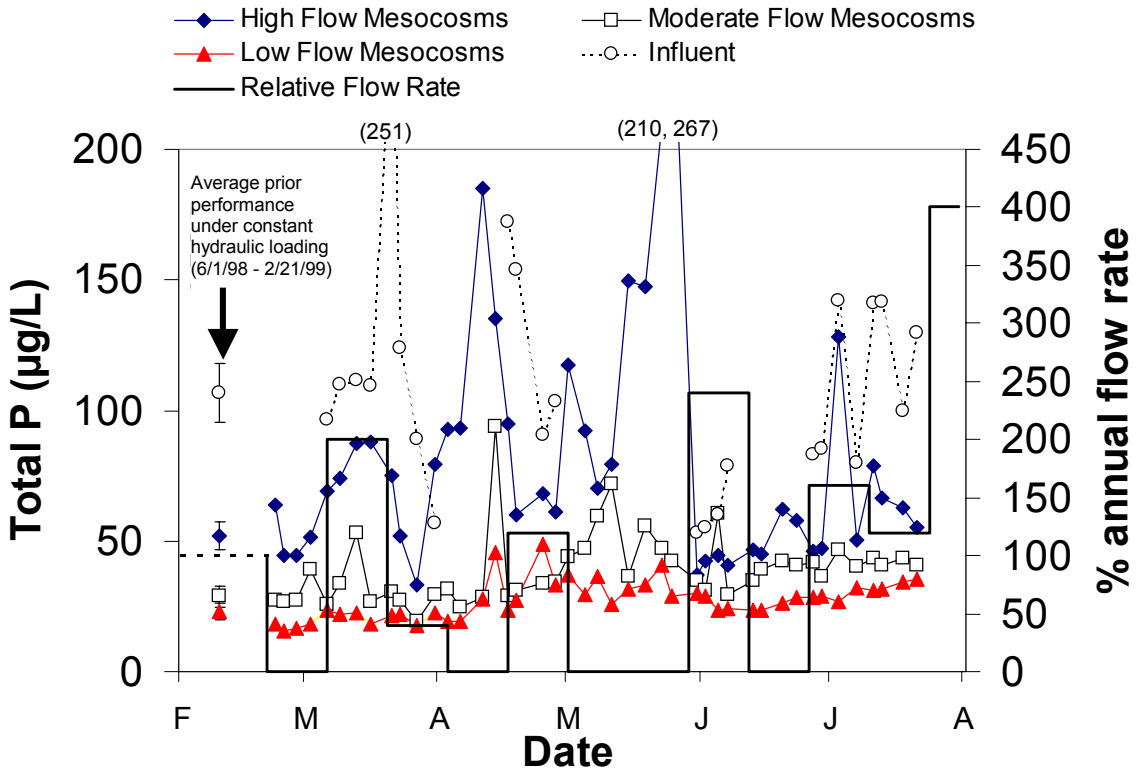

Figure 6. Mean TP concentrations in mesocosms (n = 2) operated under high, moderate and low flow rates, adjusted biweekly in a 5:2:1 ratio, respectively, with average annual loading rates of 55, 22, and 11 cm/day. Prior performance (mean ± 1 s.d.) under constant loading is indicated on the far left.

Table 3. Total phosphorus concentrations (µg/L) in the effluents from pulsed (Subtask 5v) and constant (Subtask 5iv) loading mesocosms during May-July 2000. The data for the pulsed loading mesocosms represent periods when outflows occurred and do not include *in situ* water column concentrations from no-flow periods. Pulsed loading mesocosms (two replicates per treatment) were sampled twice weekly; constant loading mesocosms (one mesocosm per treatment) were sampled weekly. n = number of samples.

SAV Loading		High Loading	Moderate Loading	Low Loading
Pulsed	Mean	**58**	**41**	**30**
(n=12)	Min	37	30	24
	Max	128	61	36
Constant	Mean	**52**	**37**	**28**
(n=12)	Min	38	22	22
	Max	87	59	56

It appears from this initial data set that conditions favoring phytoplankton populations may be created during periods of no hydraulic loading. The higher loaded mesocosms (i.e., 1.5-day HRT; HLR= 55 cm/day) seem to support higher numbers of phytoplankton, likely because they received higher historical P loadings and thus would have accreted more of a labile sediment P pool. However, conditions promoting phytoplankton growth within the high HLR mesocosms disappear when flow is resumed. This may be related to a phytoplankton "wash-out" phenomenon, the return to more favorable environmental conditions that isolate labile sediment P, and/or the absence of diffusion-gradient limitations on P-uptake by SAV.

Sequential SAV/LR Systems (Subtask 5vi)

During the quarter, the sequential system mesocosms were providing P removals of 29% for the deeper mesocosm (0.8 m) and an additional 16% removal within the shallow (0.4 m) mesocosm. The performance of the sequential SAV/LR systems has been compromised ever since the *Chara* died out in the deep SAV mesocosms, especially in replicate 1. An attempt on May 23 to restock the mesocosms with *Najas* was unsuccessful, but a later inoculation in July was successful.

Chara has the tendency, over time, to produce a thick canopy, which can occlude light penetration to the entire water column except for the upper few centimeters. We believe this condition triggers large-scale senescence of the plant which begins in the aphotic zone. The destruction of the underlying biomass of the macroalga initiates the growth of filamentous algae, which further solidifies the *Chara* canopy and renders the water column anoxic. A combination of anoxic conditions and decomposing *Chara* results in P being internally loaded to the mesocosms. It is this combination of internally loaded P and compromised treatment of the externally loaded P that results in a mediocre P removal.

We therefore decided to use this opportunity to reconfigure the sequence of treatment, adding a limerock barrel between the initial, deeper mesocosm, and the subsequent shallow mesocosm. Additionally, we removed ~ 0.3 m of muck substrate from the shallow mesocosm to normalize the substrate thickness to ~ 0.2 m for both mesocosms in the treatment train. We also increased the water depth from 0.4 m to 0.6 m. Subsequently, *Najas* was inoculated into the shallow

mesocosms. We discontinued our bi-weekly TP monitoring of the sequential treatment train to allow the *Najas* inoculum to acclimate.

Phosphorus Removal Efficiencies within Cattail- and SAV-Dominated Mesocosms

Wetlands dominated by emergent macrophytes such as cattails may function differently than wetlands dominated by SAV, since SAV can enhance both photosynthesis and nutrient uptake within the water column. An increase in water column photosynthesis leads to an increase in daytime pH, which in turn can result in a chemical immobilization of P in hard waters by coprecipitation with calcium carbonate. As part of this study, we are testing the hypothesis that P removal is more efficient in a SAV community than in a cattail community when the inflow waters have high hardness concentrations.

Beginning in mid-February, we sampled synoptically the influent and effluent waters (one grab from each location) of the two cattail and three shallow depth SAV mesocosms. In order to make comparisons between the relative P removal efficiencies as valid as possible, we selected mesocosms representing the two types of plant communities, which had equivalent water column depths (0.4 m), HLRs (10 cm/day), and HRTs (3.6 days). Weekly grab samples from both sets of mesocosms were composited over a two-week period before being analyzed for SRP, TSP, TP, alkalinity, and calcium levels. The number of replicate shallow depth SAV mesocosms was reduced from 3 to 1 on March 28, 2000, which was the date when SD-1 and SD-3 mesocosms were subjected to fluctuating water depths under Subtask 5ii. Thus only the SD-2 mesocosm, which served as a "depth" control (constant water depth maintained at 0.4 m), was used in the comparison with the cattail mesocosms.

A comparison of the total P concentrations in the effluents of the cattail and SAV mesocosms indicate a consistent pattern of superior P removal by SAV since January 1999 (Figure 7). We ascribe the difference in P removals to more underwater photosynthetic activity by the SAV community than by the emergent *Typha* community. Besides shifting the pH to higher (and more towards $CaCO_3$ supersaturation) values, the SAV community can also more directly take up P because or their underwater habit.

The quarterly data indicate that the P removal efficiencies for all three P fractions (SRP, TSP, and TP) were higher for the SAV mesocosm than either of the two replicate cattail mesocosms (Table 4). In addition, the pH was higher, and the alkalinity concentrations lower, for the SAV community, indicating that more calcium carbonate precipitation was occurring within the SAV system. Although more data are needed before definite conclusions can be drawn regarding the relative P removal efficiencies of cattail and SAV communities, it appears from this data set that SAV wetlands exhibit superior P removal processes.

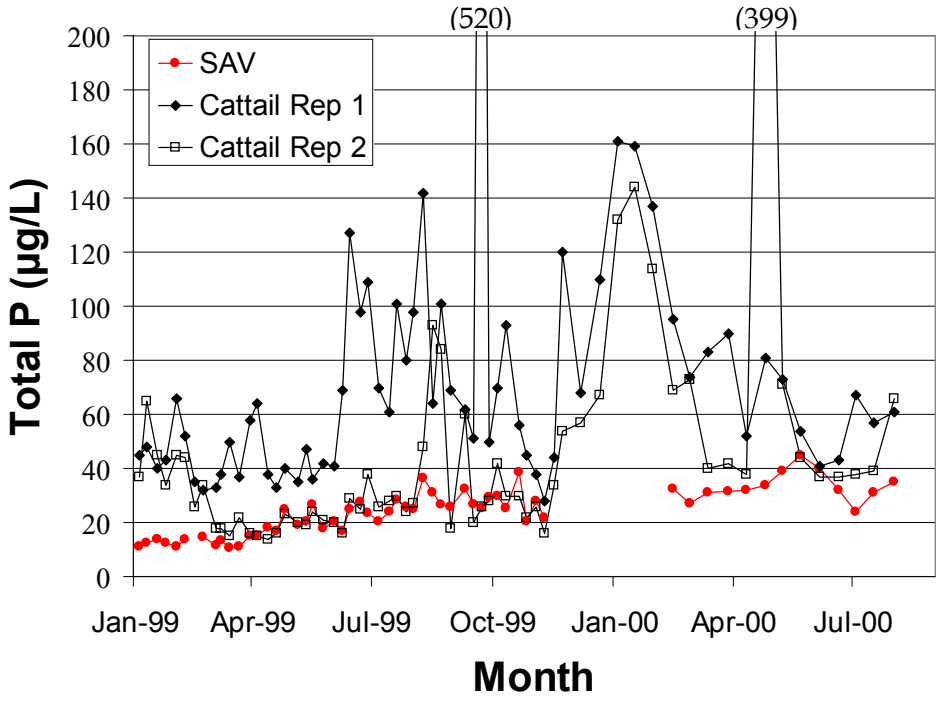

Figure 7. Total P concentrations in the effluents of shallow depth (0.4 – 0.5 m) mesocosms dominated by cattail and SAV communities. The number of replicate SAV mesocosms was reduced from three to one on March 28, 2000.

Table 4. Comparisons between one SAV- and two cattail-dominated mesocosms at the NATTS in the removal of SRP, TSP, TP and alkalinity during the May-July 2000 period of record. The inflow station represents the average of two samples - one collected from the cattail mesocosms and one from the SAV mesocosm. The data represent two-week composites of weekly grab samples.

Station	pH	SRP (µg/L)	TSP (µg/L)	TP (µg/L)	Alk (mg $CaCO_3$/L)	Sp Cond (µmho/cm)
Inflow	7.92	54	66	95	157	684
Cattail Effluent						
Rep 1	7.47	17	34	57	163	704
Rep 2	7.69	10	23	47	156	706
SAV Effluent	9.32	3	20	35	107	612

Shallow, Low Velocity SAV/Periphyton/Limerock Systems (Subtask 5vii)

During May 2000, influent TP concentrations to the SAV/periphyton systems at the SATT site, which previously had exceeded 40 µg/L, began to decline. Concurrent with this decline in inflow TP levels, P removal performance of the shallow raceways began to improve (Figure 8). From May through July 2000, the raceway and limerock effluents averaged 15 and 13 µg TP/L, respectively. The earlier, poorer performance of this system during February – April 2000 was likely caused by higher TP loadings, both from increased inflow concentrations and doubling of the hydraulic loading rate (HLR).

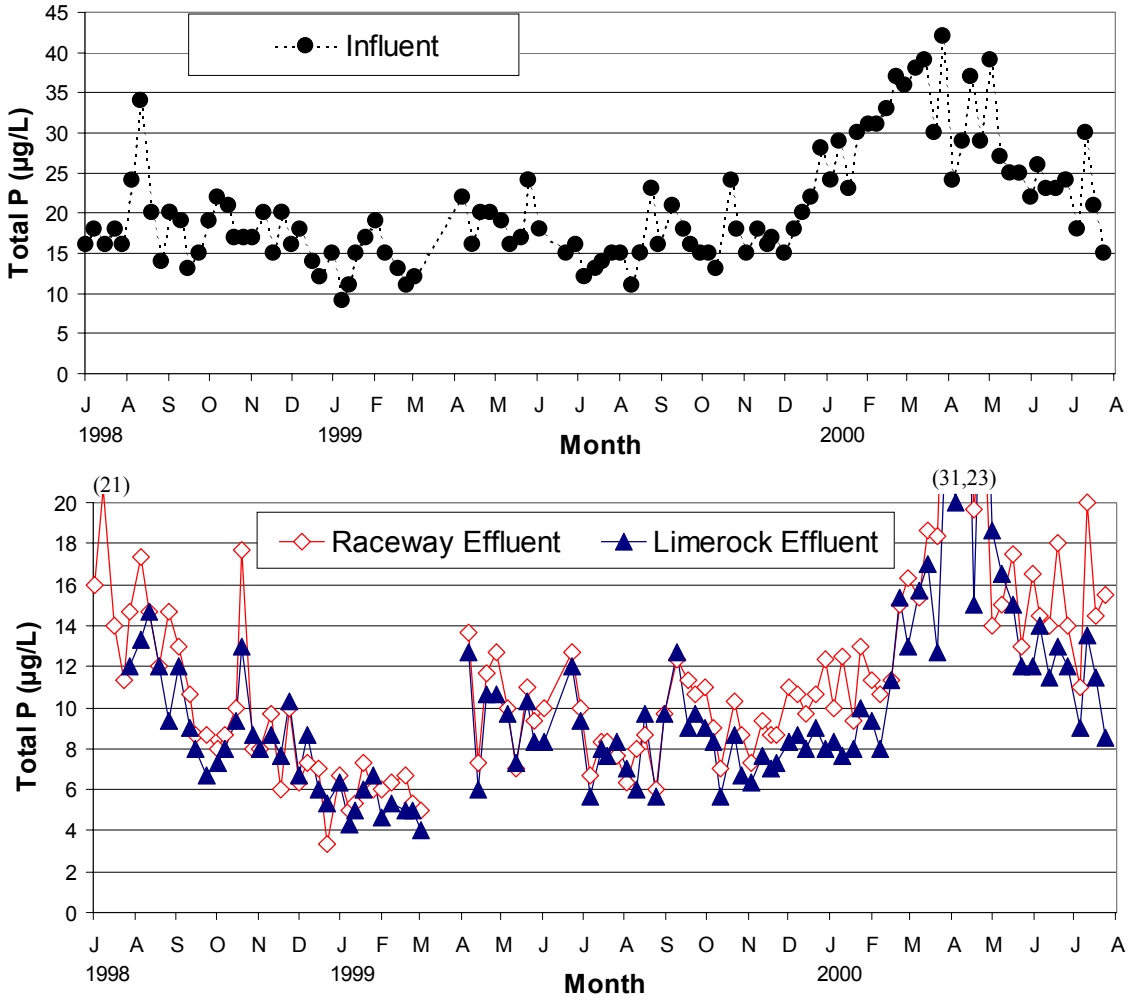

Figure 8. Total P concentrations in the influent and effluent of shallow, low velocity SAV/periphyton raceways, and in the effluent of the subsequent limerock beds. Each value represents the mean of three replicate raceways and limerock beds.

During this quarter, *Chara* began to colonize more of the influent region, and is currently the dominant vegetation in the first 15% of these raceways. This represents a shift away from *Cladophora* dominance; the middle and effluent regions still appears to be comprised of cyanobacteria (e.g. *Scytonema*, *Schizothrix*).

Growth of SAV in Post-STA Waters on Muck, Limerock, and Sand Substrates (Subtask 5ix)

In an effort to understand the interaction between HLR and different substrates on P-removal performance by SAV, we reduced the HLR to the sand, muck, and limerock substrate mesocosms on several occasions during the past six months (Figure 9). Twice last quarter and again on May 23, 2000, the flows were reduced by 50%. Each treatment train now has an HRT of 10.4 days.

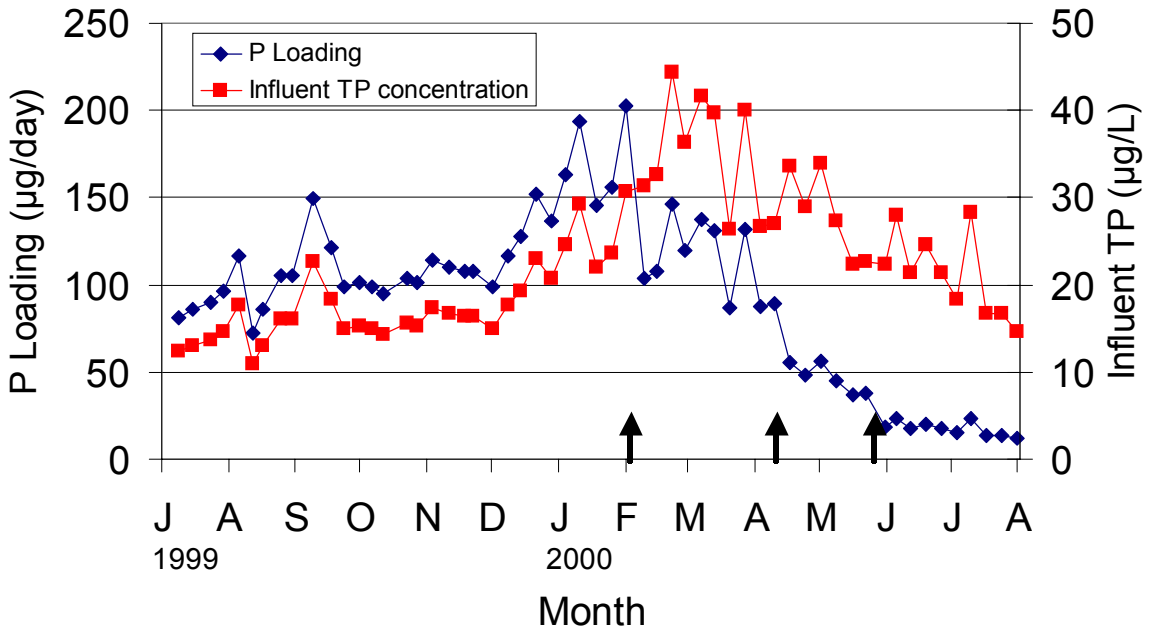

Figure 9. Influent TP concentrations and P loadings to the substrate mesocosm experiment. The arrows denote times when HLR was successively reduced 50%.

During this third quarter, mean system outflow TP concentrations were lower from the limerock (17 µg/L) than the muck (20 µg/L); sand exhibited the highest effluent TP (27 µg/L) (Table 5). This is contrary to trends described in earlier reports (DBEL 2000), where the muck

substrate mesocosms provided lowest outflow TP concentrations (Figure 10). Although the SAV biomass is sparse in the limerock substrate mesocosms, they have performed as well as (or better than) the muck substrate mesocosms, which do support dense SAV populations. Clearly, removal mechanisms other than SAV uptake are probably occurring within the mesocosms. These mechanisms include microbial uptake (bacterial and nanoplankton), enzymatic P hydrolysis, adsorption, photolysis and particle P sedimentation. Moreover, the limerock substrate may perform a role important for P removal by maintaining alkalinity levels in the water column (P coprecipitation with $CaCO_3$) or by creating an environment amenable to refractory sediment-P accretion.

Table 5. Mean total phosphorus concentrations (µg/L) in the influent and effluents of duplicate substrate mesocosms during May-July 2000.

	Influent	Limerock	Muck	Sand
Mean	23	17	20	27
Max	35	30	28	44
Min	15	12	13	17

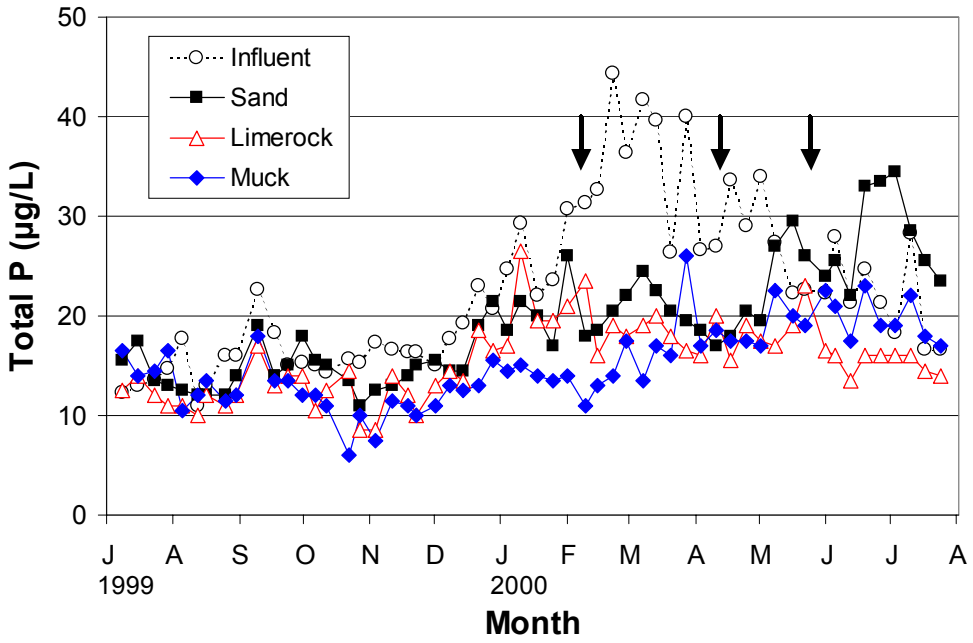

Figure 10. TP concentrations in the influent (n = 3) and effluents (n = 2) of SAV mesocosms established on limerock, muck and sand substrates. Each arrow represents a 50% reduction of flows.

Effect of Filter Media on P Removal Performance (Subtask 5x)

DBEL's initial work with the SAV/LR concept at the North and South Advanced Treatment Technology Sites demonstrated that the LR bed is serving principally as a physical filter for particulate P. During this follow-on study, DBEL and HSA personnel will screen other filter media in addition to limerock. We have made our final selections of four different filter media within various size ranges (Table 6). One of the four media types (quartz sand or pebbles) will serve as a control (i.e., inert) medium. The other three media were selected for their potential P-removal capabilities. Pro-Sil Plus™ is a commercially available soil-liming material that is processed from Ca and Mg silicates. It is reported by the distributor to have a high P adsorption capacity. Iron-coated sand was collected from a construction site in central Florida. The last filter medium, limerock, was obtained from the West Palm Beach Aggregates quarry across from the STA-1W on Southern Blvd.

We constructed sixteen PVC columns (20.3 cm ID) at the South Advanced Treatment Technology Site (SATTS). Post-STA (low particulates, low SRP) waters will be fed through three SAV tanks (4.66 m long x 0.79 m wide x 1.00 m deep) with a water column depth of 74 – 79 cm and a HLR of 117 cm/day, and then gravity fed to the packed (56 cm deep) columns. The experiment will last 13 weeks, after which the assembly will be moved to the NATTS for another 13 weeks of study with Post-BMP waters.

Table 6. Media types and sizes to be used in the Filter Media Experiment.

		Filter Media Type			
Sieve No.	Size Range	Quartz Sand	Limerock	Pro-Sil Plus™	Fe-coated Sand
<No. 3 >No. 6	**Coarse** 3.35-6.86 mm	✓	✓	✓	
<No. 6 >No. 10	**Medium** 2.00-3.35 mm	✓	✓	✓	
<No. 20 >No. 60	**Fine** 0.25-0.85 mm	✓			✓

Particulate Phosphorus and Dissolved Organic Phosphorus Characterization and Stability (Subtask 5xi)

Findings from DBEL's Phase I research demonstrated that the effluent from both SAV and LR unit processes consists largely of particulate P (PP) and dissolved organic P (DOP). The ability of a supplemental technology to achieve the 10 ppb TP target depends on its ability to reduce these fractions to extremely low levels. The capabilities of the supplemental technologies to "treat" these P species will vary, depending on the size fraction, stability, and transportability. It would be helpful in the design of STAs to understand whether particles exported from a SAV or cattail unit process are the same particles in the inflow source water, or if they are generated internally in the wetland. Understanding the differences, if any exist, of particle type, size and zeta potential (the potential difference between the plane of shear and the bulk phase), will provide insight on the transport potential of those particles.

During this past quarter, DBEL employed a tangential-flow filtration technique to concentrate particles in waters at various stages of treatment (i.e. Post-BMP, Cell 4 effluent, test cell effluent). This technique allows the processing of large sample volumes while at the same time minimizing the artifacts from concentration polarization that are observed with typical stirred-cell filtration devices (Cooper et al. 1999).

On July 5, 2000, DBEL and HSA personnel performed a practice run using the Consep Tangential Flow Filtration module and a Setec peristaltic pump. The sample water was a composite of Cell 4 inflow (volume-weighted equally from surface waters collected at culverts A, F, I). We selected polycarbonate filters (Osmonics) with pore sizes equal to the size fractions (0.40 and 0.05 μm) we believed would show the greatest differences in enzyme hydrolysis and SRP releases under low pH and redox conditions. A diagram of the sequential filtration procedure is shown in Figure 11. During the practice run, we familiarized ourselves with assembly and disassembly of the filter unit, operating conditions (i.e., pump speed and pressures across and through the membrane filter), and the times required to achieve a reasonable concentration factor (3 – 5X) for a given volume of raw water.

The raw water, retentates (0.40 and 0.05 μm) and permeate from the 0.05 μm filter were delivered to the Biogeochemistry Lab in the Soil and Water Science Department at the

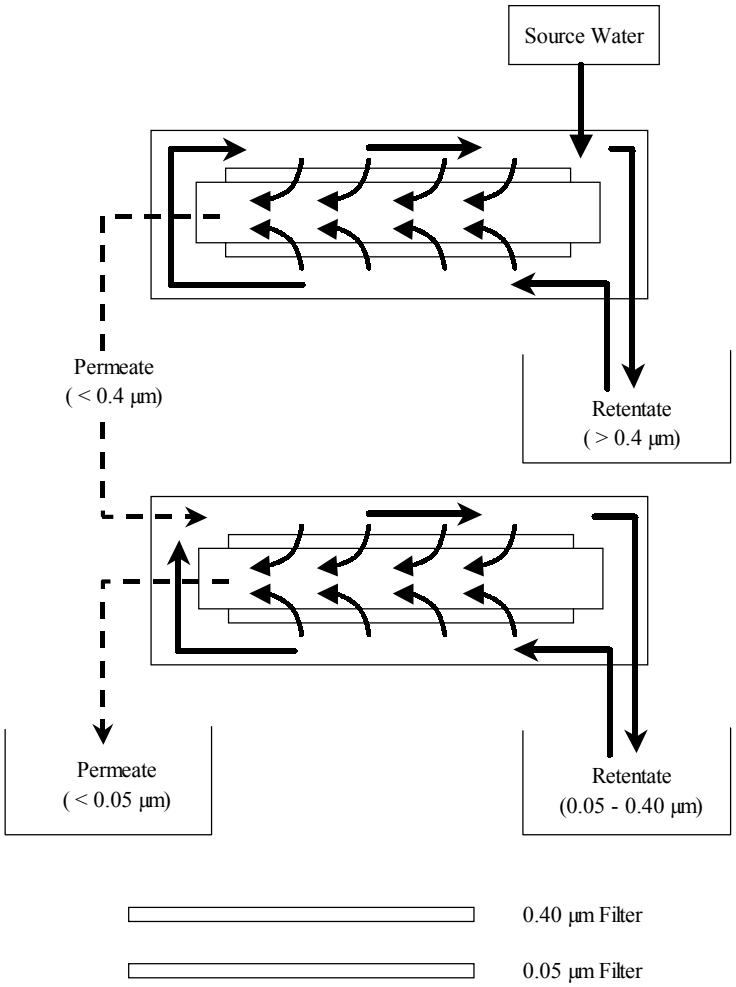

Figure 11. Sequential tangential-flow filtration procedure used in particle concentration of Cell 4 inflow water according to size ranges of 0.05 μm to 0.40 μm.

University of Florida for analysis of SRP, TSP, and TP concentrations. TP and TSP samples were preserved with H_2SO_4 to a pH<2 and SRP samples were frozen after filtering through a 0.45 μm syringe filter. Based on the P concentrations in each of these fractions, the proposed incubation time (6 hr), and the purity of the enzyme source, the amount of each enzyme to be added for optimum enzyme activity to insure substrate saturation can be estimated. Beyond the P analyses, no other analyses or experimentation were performed for this "practice run".

We performed tangential flow filtration on a second Cell 4 inflow composite sample (38 L) again on July 18 – 24 with the aim of collecting adequate sample retentate and permeate (5 – 6.5 L for each size fraction) for the experiments described below. All particle fractions and the raw waters were kept on ice during the 7-day concentration process. The volume of the raw water

was concentrated 6.0 fold in the 0.40 µm size retentate; the permeate from the 0.40 µm pore size filter was concentrated 4.8 times (or 6.0 times with respect to the raw water volume) in obtaining the 0.05 µm size retentate.

The following experiments were performed at the Biogeochemistry Lab at the Soil and Water Science Department:

1) Enzyme Hydrolysis

The following enzymes were tested for their capacity to hydrolyze SRP from dissolved and particulate compounds at a pH = 8.0.

- Phytase
- Alkaline Phosphatase
- Phosphodiesterase
- Alkaline Phosphatase + ATPase
- Alkaline Phosphatase + ATPase + Phytase + Phosphodiesterase

2) Low pH Conditions

A lower than ambient pH of each retentate, the permeate, and the raw water were attained by bubbling air with 0.3% CO_2 into each incubation vessel. The control vessels received air-only, which contains 0.03% CO_2.

3) Low Redox Conditions

Reducing conditions were maintained by bubbling N_2-only gas through each retentate, the permeate, and the raw water incubation vessels. Another set of treatment vessels received a N_2 + 0.3% CO_2 mixture (for comparing the interactive effects of a low pH and low redox on SRP release). The vessels receiving the air-only served as the controls.

The incubation period for the Enzyme Hydrolysis experiments is 6 hours; 20 days incubation were used for each of the Low pH and Low Redox experiments. All treatments and controls were performed in triplicate. Data from this experiment will be provided in the next quarterly report.

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Task 6. Test Cell Studies

Herbicide Application and SAV Stocking

During the month of June, water levels in the south test cells (STC-4 and STC-9) were drawn down in an effort to eliminate *Hydrilla*, so that other macrophytes, including *Najas*, could be established. A topical herbicide (Rodeo®) was sprayed on emergent plants including *Typha* sp. (cattails), *Panicum repens* (torpedo grass) and *Echinochloa* sp. (barnyard grass). Afterward, the water level in each of the south test cells was lowered to expose the muck substrate, and another herbicide, Endotholl®, was applied to discourage regrowth of *Hydrilla* tubers. After two weeks, the water level was brought up to 0.4 m in both STC-4 and STC-9. After another two-week equilibration period, *Najas* was harvested from the northeast corner of Cell 4, and stocked into STC-4 and STC-9 at approximately 0.9 kg wet/m^2. *Ceratophyllum* (~1 metric ton) was added to the influent region of NTC-1 on July 11.

Limerock Berm Head Loss Assessment

In May 2000, limerock berms were constructed across the width of the cell in the effluent region of NTC-15 and STC-9, to provide particulate P filtration at the back end of the test cells. Results from mesocosm-scale experiments (Subtask 5iv) suggest that these berms will trap particulate P, which ultimately may partially transform to SRP. Any SRP released in this manner will be removed by the SAV located between the berm and the effluent weir. Following berm construction, we conducted head loss measurements in NTC-15 and STC-9. This was performed by increasing the influent aperture to allow maximum flow through the cell, and measuring the water stage on either side of the berm. The berms caused no measurable difference ($\pm$ $^1/_{100}$ foot) in stage for either NTC-15 or STC-9.

Water Quality Monitoring

Following the head loss measurements, influent apertures were returned to 1 inch. Each of the four test cells now receive a HLR of (5 cm/day), and are maintained at a constant water depth of 0.4 m. The north test cells' influent and effluents are currently composited (5 daily grabs/week) for TP, TSP, SRP, dissolved calcium, alkalinity, and specific conductance.

Temperature and pH are measured daily. Water quality monitoring will resume in the south test cells in July of this year.

The two north test cells continue to remove nearly all SRP (effluent, $\bar{x}$=3 µg/L) from the influent waters (range of 19-106 µg SRP/L, $\bar{x}$=60 µg SRP/L). Test cell NTC-1 was not performing as well as NTC-15 with respect to TP effluent concentrations until the resident alligators were removed in late June and additional *Najas* and *Ceratophyllum* inoculum was added during July. The most recent water quality data shows an improvement in removal performance in NTC-1 (Figure 12).

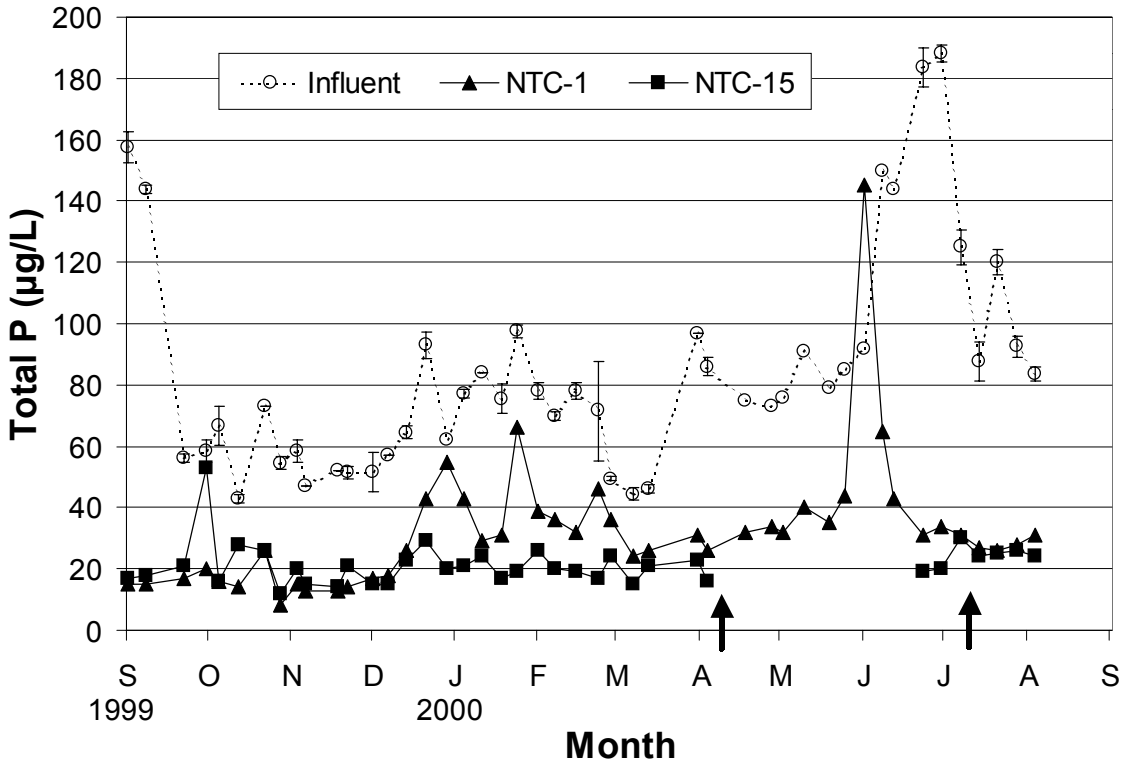


Figure 12. Total P concentrations in influent to and effluents from north Test Cells #1 and #15. The first arrow denotes an interruption in water quality monitoring for the installation of a limerock berm in NTC-15; monitoring was resumed June 23, 2000. The second arrow denotes the addition of ~ one metric ton of *Ceratophyllum* to the influent region of NTC-1. Error bars = ± 1 s.d.

Task 9. Cell 4 Experiments

Sediment P Stability and Characterization

We initiated work on this task in June 2000, using sediments collected from the inflow region of Cell 4. We are scheduled to repeat the same P-fractionation scheme and P stability/release treatments on sediments collected from the southern region (near the outfall) of Cell 4 in July.

Three stations adjacent to the northern levee of Cell 4 were cored (10.1 cm ID aluminum corer) on June 14th. The average depths of the marl were 7, 14 and 15 cm for the three stations. One core from each station was kept separate for the fractionations, while the remaining 7 cores from each station were composited as a homogeneous substrate deployed in the stability/release experiments. A blanket of nitrogen gas was applied to the headspace of the composite container and to the zip-lock bags holding the sediment for P fractionation.

Methodology for fractionations of organic and inorganic P in Cell 4 sediments

Sequential P fractionations were conducted on three distinct sediment samples collected at each of the three sampling stations in the northern section of Cell 4. The inorganic-P fractionation scheme followed a methodology suitable for calcareous sediments (Hieltjes and Lijklema 1980). Briefly, 5 grams of wet sediment are exposed to the following extractants (40 mL) on a sequential basis: 1M NH_4Cl (pH adjusted to 7.0) for two 2 hr extraction periods; 0.1N NaOH for 17 hr; and 0.5 N HCl for 24 hr. Analysis for SRP (filtered with Whatman™ 0.45 μm) within each extraction, and TP (filtered through Whatman™ 41) on only the 0.1N NaOH extraction, yield the fractions of P in the porewater, loosely sorbed, and $CaCO_3$-associated (NH_4Cl), iron and aluminum bound-P (NaOH-SRP), biogenic-P (NaOH-TP minus NaOH-SRP), and the Ca mineral-P (HCl).

Fractionations of the organic P (P_o) pool were performed (Ivanhoff et al. 1998) on triplicate sediments from each sampling station as well. Soil samples (5 g wet) are exposed to chloroform (2 mL) prior to the following series of extractants, each of which produces a residue that is filtered (Whatman™ 41) and analyzed for TP: first, 0.5 M $NaHCO_3$ at pH = 8.5 for 16 hr, then 1.0 M HCl for 3 hr, and finally 0.5 m NaOH for 16 hr. An aliquot of the supernatant is acidified to pH 0.2 and centrifuged for 15 min at 2800 rpm. The supernatant is then analyzed for TP, which

provides the fulvic acid (FA) fraction of P_o. The humic acid (HA) P_o fraction is obtained by subtracting the FA fraction from the TP concentration in the NaOH-extracted residue prior to centrifuge.

Additionally, the sample (5 g wet weight) is exposed to 0.5 M $NaHCO_3$ for 16 hr without the chloroform pretreatment. Total P in the residual of this last extraction is subtracted from the TP concentration in the residual from the first extraction (chloroform/$NaHCO_3$ at pH = 8.5) to calculate the microbial P fraction.

Methodology for sediment stability under low/high calcium and low/high SRP conditions, and under low pH and redox conditions

Sediments from the Cell 4 inflow region were subjected to a range of environmental conditions to assess stability of the associated P. The following eight treatments including controls were each replicated in triplicate:

1. 3% CO_2 in air to induce a very low pH
2. 0.3% CO_2 in air to induce a moderately low pH
3. 0% CO_2 in air (CO_2-free) to induce a high pH
4. 0.03% CO_2 in N_2 to lower the redox potential
5. Air-only + Ca spikes (50 mg/L) to provide hard water
6. Air-only + SRP spike (125 µg/L) to simulate increased ambient P concentrations
7. Air-only to serve as a control for the pH and redox altered sediment, and the Ca/alk and SRP spiked sediment
8. Air-only upon reflooding of desiccated (48 hr drying time) sediment

Incubations began on June 15, 2000, the day following field collection of the composited sediment. Each 500-mL incubation flask received 100 mL of wet sediment plus 400 mL of Cell 4 outflow water, which had been collected the same day (June 14) as the sediment coring. The gases were sparged through distilled water prior to being gently bubbled (8-10 mL/min) into the water column of each incubation vessel. Frequent redox and pH measurements within each vessel permitted adjustment of the gas flows for maintaining targeted redox and pH values.

Incubations were performed in the dark, within a water bath held at room temperature. The minimum and maximum temperatures during the entire incubation period (12 days) were 20 and 22°C, respectively.

Samples were collected at the beginning and end of the 12.6-day study period from each incubation vessel for the following analyses: SRP, TSP, alkalinity, calcium, specific conductance, pH, redox potential, and iron. Additionally, sampling for SRP, pH, and redox potential was conducted on a more frequent basis, occurring after 0.3, 0.7, 1.5, 3.0, and 6.0 days in addition to the initial and ending times. Sediment bulk density was measured prior to incubations.

Initial Results of Sediment Stability and Characterization

The initial (t = 0) pH ranges for the four pH treatments were 7.58 to 7.79 for the inflow sediment and 8.06 to 8.13 for the outflow sediment.

The effect of pH on the release of SRP from inflow sediment was negligible among three higher pH treatments (0.3, 0.03, and 0.0% CO_2 provided average pH values of 7.76, 8.42, and 8.88, respectively) for the first 36 hours (Figure 13). After an elapsed time of 72 hours, the SRP release began to show a pH effect in the 0.3% CO_2 treatment (mean pH of 7.76). In contrast, the lowest pH treatment (3% CO_2 with mean pH = 7.01) yielded an immediate and significantly higher release rate than the three higher pH treatments (Figure 13).

All the pH treatments exhibited a common release pattern in that SRP release was highest during the first 16 hours, and then gradually decreased beyond that time (Figure 13). For the high pH treatment (0.0% CO_2), the SRP release became negligible during most of the incubation period, while in the remaining treatments the release continued until the end of the incubation period, albeit at a reduced rate. These lower release rates during the latter 90% of the incubation period compared to the first 36 hours can be due to one or more of the following phenomena:

1) The SRP released initially into the water column reached an equilibrium with the remaining P in the sediment, whereby the law of mass action prevented a further net release of SRP.

2) The available pool of sediment P simply became exhausted first at the higher pH treatments.

3) The initial high SRP release rates are associated with a more labile sediment P pool which is exhausted quickly, whereas the longer, lower rate of SRP release is attributable to a separate less labile, and perhaps larger sediment P pool.

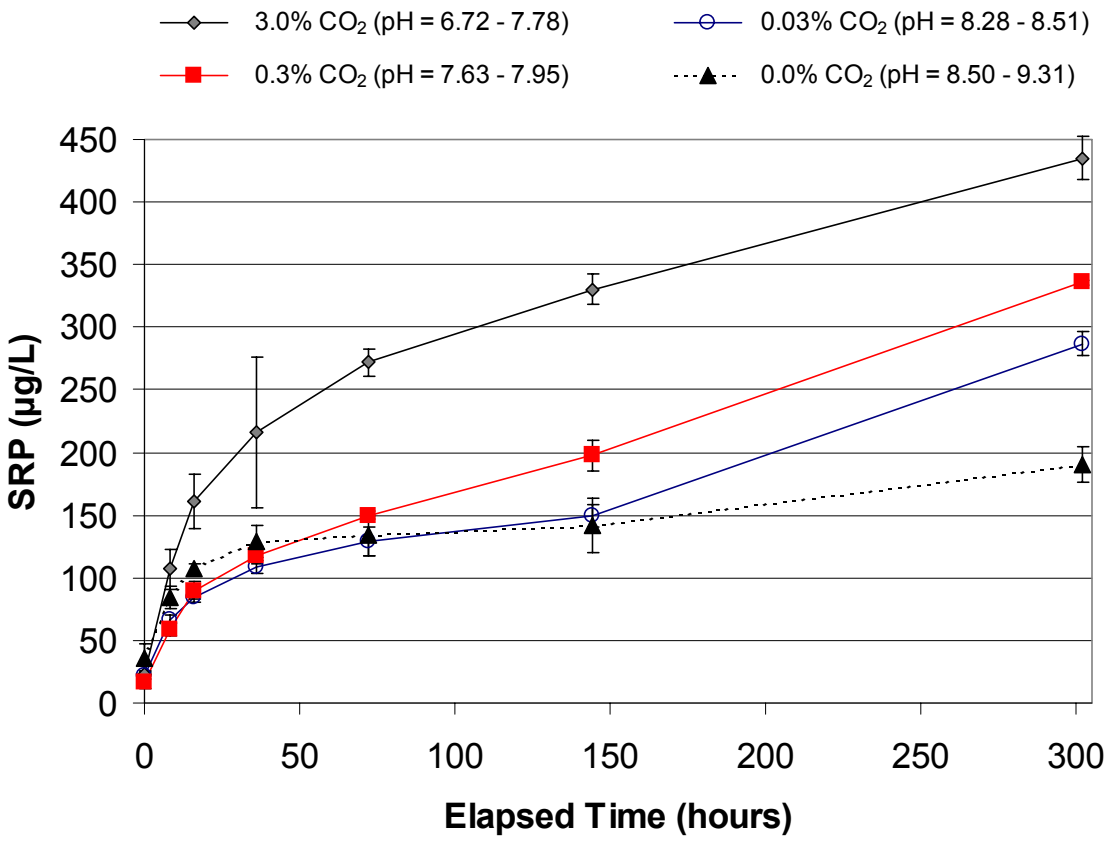


Figure 13. Time course for SRP release from Cell 4 inflow region marl sediments exposed to varying pH values. Each data point represents the mean ± 1 s.d. of 3 replicates.

Which of the above explanations is responsible cannot be defined without further experimentation, although the data from the P fractionations indicates that there were still adequate reserves of labile P pools (e.g., NH_4Cl-P) in the post-incubated (12.6 days) sediment (Figure 14). However, the data unequivocally demonstrate that both the rates and extent of SRP release from the marl is affected by the pH of the ambient water in an inverse and linear manner

(Figure 15). After a 302-hour exposure period, 120 µg SRP/L can be released for every decrease in pH of one unit.

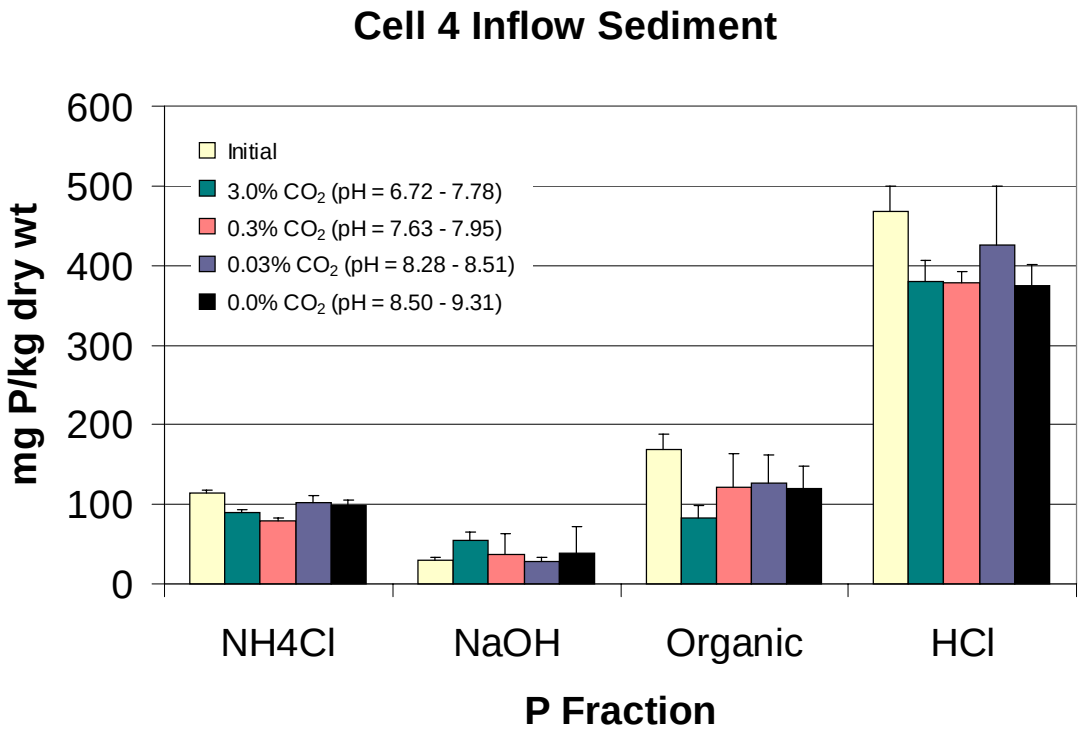


Figure 14. Inorganic P fractionation of Cell 4 inflow region marl sediments before (Initial) and after incubations at varying levels of pH for 12.6 days. The bars above the histogram columns represent + 1 standard deviation.

Based on the inorganic P fractionations of pre- and post-incubated sediment, there are three possible sources of P that contributed to the release under low pH exposure. The differences between the initial and post-incubated NH_4Cl-P, HCl-P, and organic-P fractions indicate that all three of these pools could have contributed P since they all lost P during the incubation period (Figure 14). Most of the decrease in sediment P occurred in the HCl-P and organic-P pools, indicating that relative to the more labile NH_4Cl-P and NaOH-P, the less labile organic-P and HCl-P pools are the major sources of the released P. Considering the high calcareous nature of the sediment and the known effect that pH has on $CaCO_3$ solubility (Stumm and Morgan 1981), it is likely that the inverse relationship between pH and SRP release observed in the data set (Figure 15) is due to the desorption from, and dissolution of, $CaCO_3$.

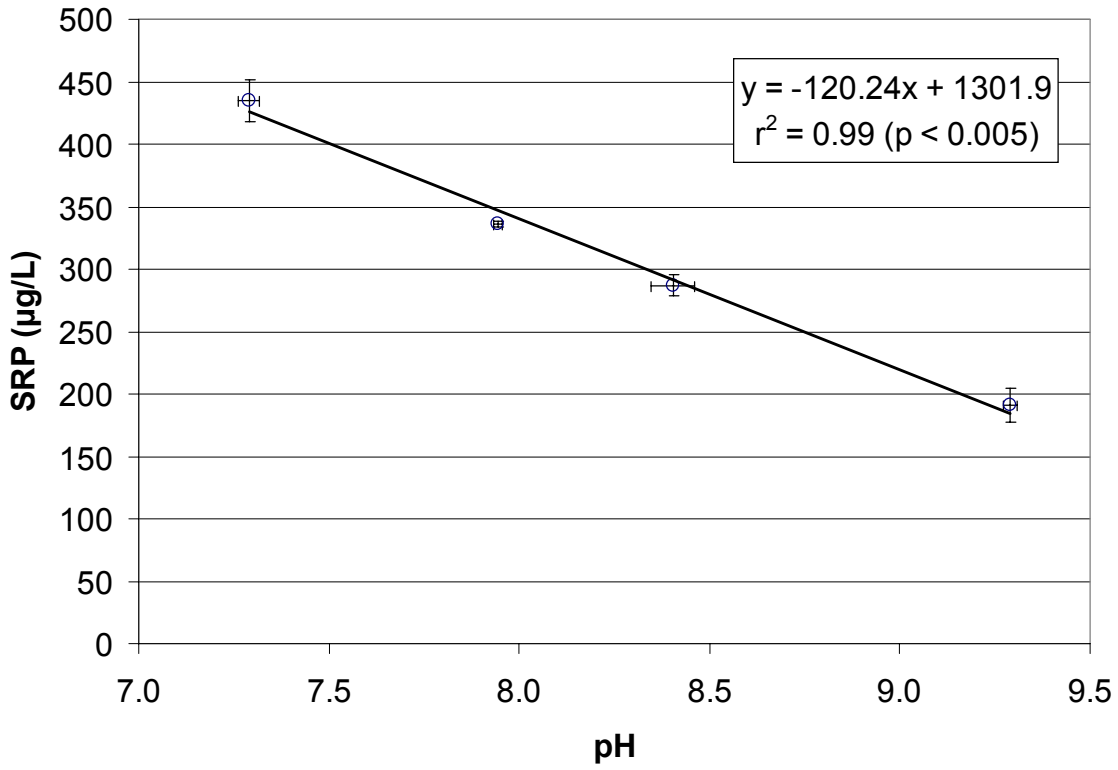


Figure 15. The effect of pH on the release of SRP from marl sediments composited from three locations in the inflow region of Cell 4. The incubation period was 302 hours (12.6 days). Each data point represents the mean ± 1 s.d. of 3 replicates.

Anoxic conditions (average E_{h7} = +114 mV) produced an immediate and extended response in SRP release compared to the oxic control (average E_{h7} = +495 mV) (Figure 16). This indicates that there are significant quantities of Fe-associated P in the marl, or bacteria containing surplus P are releasing it during anaerobic conditions (Gächter and Meyer 1993).

For the oxic vs. anoxic treatments, there were no discernible differences in their P fractions at the end of the 12.6-day incubation period and the respective initial (pre-incubation) fractions (Figure 17). Theoretically, a decline in the NaOH-P fraction should have occurred for the anoxic treatment. Since it did not occur, another mechanism other then Fe reduction may have been operating, such as bacterial release (Gächter and Meyer 1993). Indeed, the organic-P pool did show a slight decrease between the initial and the end of the incubation period, but it was not statistically significant.

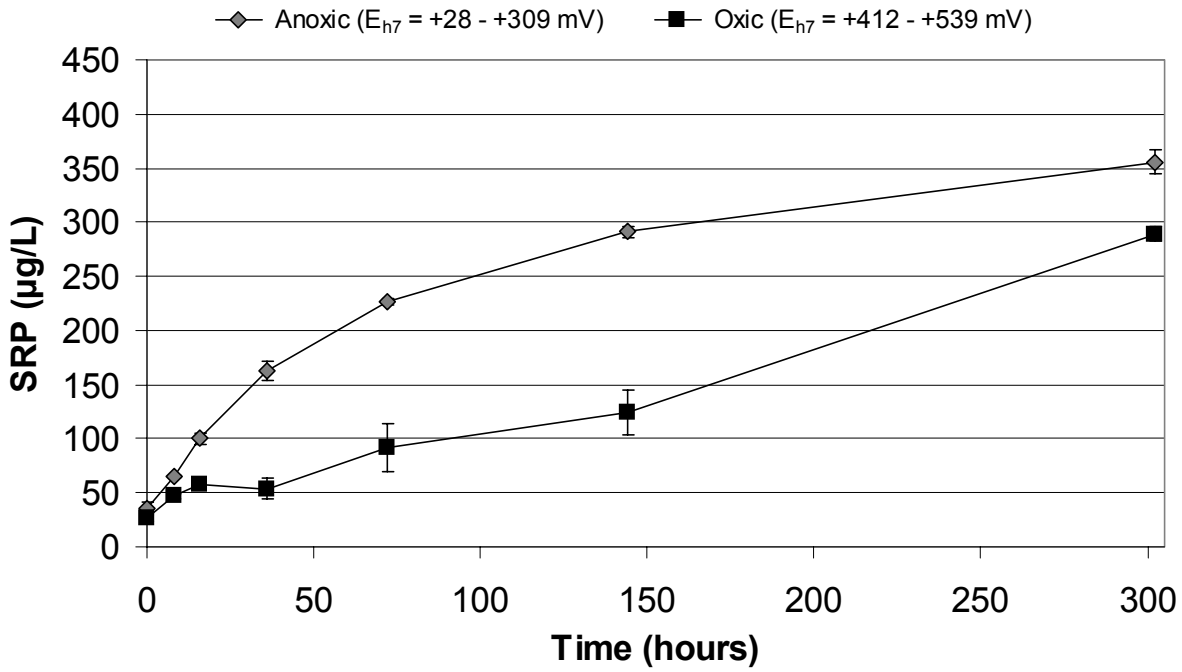


Figure 16. Time course for SRP release from Cell 4 inflow region marl sediments exposed to oxic and anoxic conditions. Each data point represents the mean ± 1 s.d. of 3 replicates.

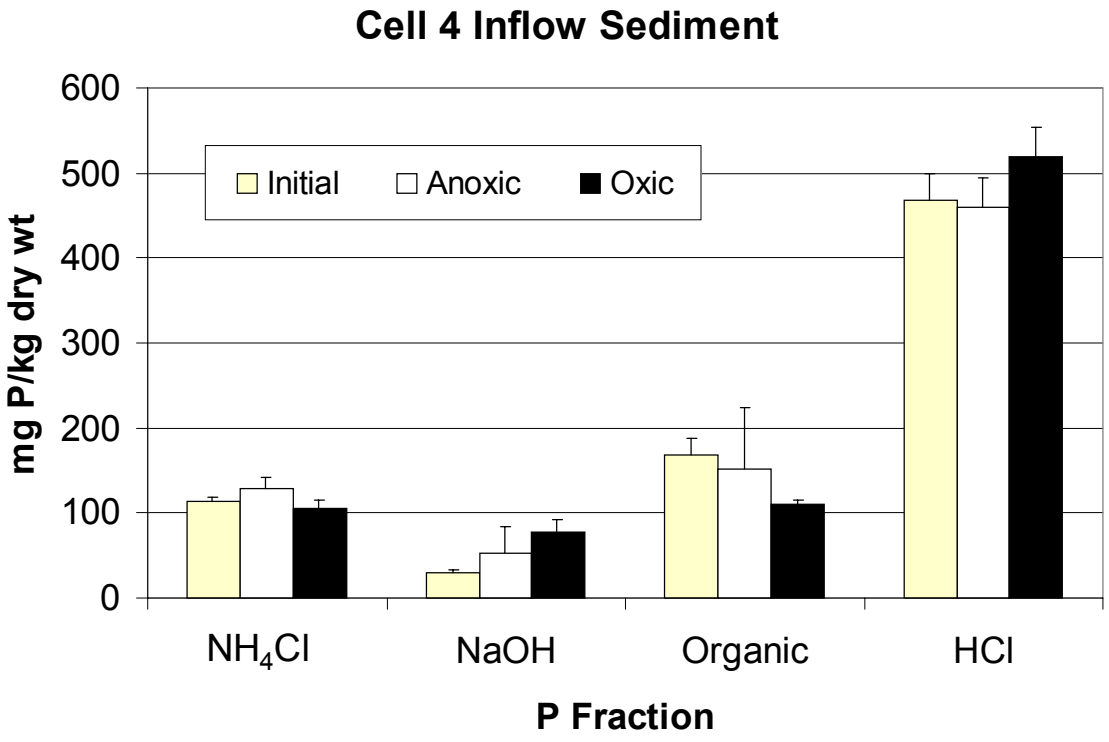


Figure 17. Inorganic phosphorus concentrations of Cell 4 inflow region marl sediments composited before (Initial) and after incubations under anoxic and oxic conditions for 12.6 days. The bars above the histogram columns represent + 1 standard deviation.

Although these data indicate significant effects by lower pH and redox conditions on sediment SRP release, the ecological implications may not be as clear-cut or pronounced. Perhaps one of the more important considerations is the length of time that a low pH or anoxic event would likely occur in the field. Our experiment lasted 12.6 days. Low pH or redox conditions typically may be of shorter duration in the field. For example, while inflow waters to our NATTS mesocosms did on occasion reach a pH of 7.0 during the day, the daytime pH of the outflow from those mesocosms was never less than 7.6. We never observed, in our more limited nighttime database for the mesocosms, a pH value less than 7.4. Simiarly, the temporal and spatial profiling of SAV mesocosms during Phases I and II usually revealed redox values above +250 mV, particularly in the surface waters.

It also is important to note that all of the sediment SRP release experiments were performed in the absence of SAV. It is very possible that a given "flush" of sediment SRP initiated by low pH or redox conditions would immediately be taken up by the SAV community, particularly if the plants were P-limited. The SRP data collected throughout our Phase I and II projects have indicated that SRP does not travel very far down the flow path of a SAV bed before it is reduced to below detection limits. Thus the presence of the SAV community may actually "buffer" the wetland from episodic sediment releases and discharges of SRP into downstream waters.

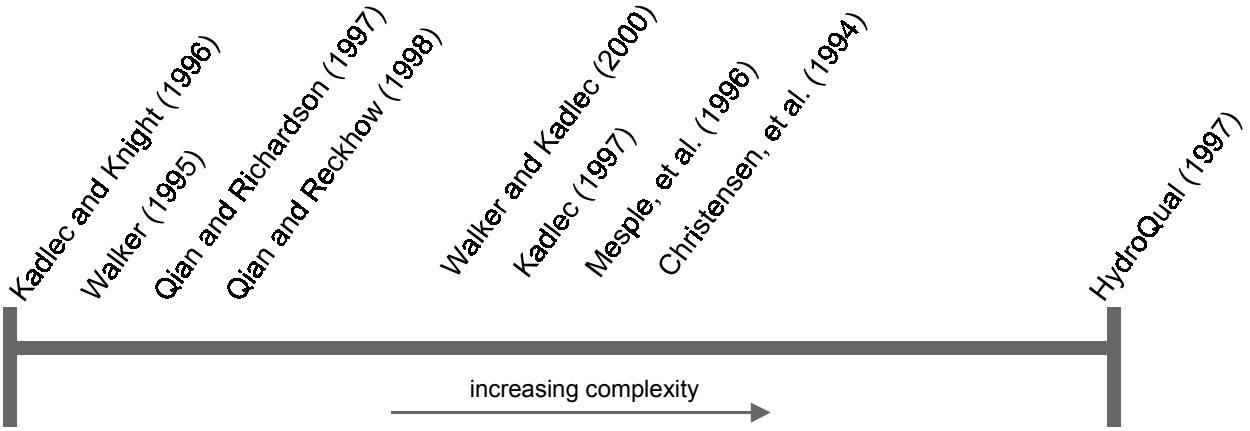
Literature Review: Modeling Phosphorus Removal in Wetlands

Background

As part of our Task 9 activities, we conducted a literature review to investigate past efforts of modeling phosphorus removal in aquatic systems. The purpose of this review was to identify the types of models that have been developed in the past and to assess the utility of various modeling features for our process model development.

We reviewed nine different approaches that have been used for modeling P removal in aquatic systems. Water quality models are commonly categorized as either empirical or mechanistic. Empirical models are based on statistical summaries of input-output relationships. Mechanistic models are based on the modeler's interpretation of the fate and transport of water quality

parameters with one or more mathematical equations (Reckhow 1994). Mechanistic models, which can also be described as 'process' models, aim for a 'correct' yet simplified interpretation (theory) of reality. In contrast, empirical models aim for accurate parameter estimation with less emphasis on process description (Reckhow 1994). In Figure 18, the nine models that we reviewed are placed along a continuum from empirical to highly articulated mechanistic models. Figure 18 can also represent a continuum of increasing model resolution and complexity. We found this continuum concept to be a helpful way to organize and relate models to one another.



Empirical Models
Single parameter
Low spatial and process resolution (I/O)
Simple hydraulic models (PFR a\or CSTR)
Steady-state, long-term average performance
Design modeling

Process Models
Multi-parameter (sometimes >200)
Process and spatial resolution
Detailed hydrodynamics
Dynamic, event-driven time histories
Analysis (research) modeling

Figure 18. Literature review of nine different approaches to modeling P removal in aquatic systems.

Empirical Models for P Removal in Wetlands

Kadlec and Knight (1996) calibrated a P mass balance model to predict long-term average TP removal based on the collective behavior of 20 individual wetland systems (a cross-sectional database). The model assumes that wetland hydraulics are described by plug flow behavior with no mixing. The model also assumes that phosphorus removal occurs in direct proportion to the in-column TP concentration; this is commonly referred to as a 1st order removal model.

The coefficient of proportionality between removal and in-column concentration is termed the TP settling rate, k, and was estimated, in this case, using a wetland's long-term average input and output TP concentrations.

As is typical of other empirical models, this model structure does not attempt to represent specific physical processes, only their net consequences. Kadlec and Knight's (1996) recommended design value for emergent wetlands, k = 12.1 m/yr, resulted from averaging individual k's calculated from 20 wetlands located throughout the United States. This relatively simple model establishes a mathematical relationship between inflow concentration, outflow concentration, settling rate, flow rate, and surface area and can be readily solved for the parameter of interest. A common criticism of models based on the collective behavior of many wetlands is that they are too general to apply to a single particular wetland design, since the represented collective behavior may not be pertinent to particular site-specific conditions (Qian and Reckhow 1998).

Walker (1995) also developed an empirical model for TP removal based on assumptions of plug flow hydraulics and first order TP removal. The model consists of a coupled water and mass balance representing long-term steady-state conditions in the wetland. Rather than calibrate to input/output data from a cross-sectional database as did Kadlec and Knight (1996), Walker calibrated to the spatial gradient of water-column and peat accretion data within a single wetland, Water Conservation Area-2A (WCA-2A). Using a least squares estimation technique, Walker (1995) demonstrated that the 26-year-average rate of peat accretion was proportional to average water column concentration, with a proportionality constant of k = 10.2 m/yr. The simplicity of the model and strength of Walker's statistical analysis (demonstrating that the 90 percent confidence interval for parameter estimation lies between 8.9 and 11.6 m/yr), led to adoption of this model as the design basis for long-term performance in SFWMD Stormwater Treatment Areas (STAs). In design mode, the model was used to size STA surface areas given average expected influent flows and TP loads and a target outflow concentration of 50 ppb (Walker 1995).

A common criticism of a design tool based on analysis of a single system is that the information could be too site-specific to be meaningfully applied elsewhere (Qian and Richardson 1997). However, Walker's model was applied only to wetland designs that shared similar climate (sub-tropical Florida), similar intended plant community (emergent macrophytes), similar influent water quality (TP enriched), and similar sediment conditions (peat) to the source wetland, so the approach may be justifiable in this case.

Qian (1997) also criticized Walker's approach on the basis that it failed to take into account the effect of uncertainty in data and model variables, that the sheet flow assumption may have been grossly violated, and the data set was arbitrarily truncated. Qian (1997) employed estimation techniques that he argued were more appropriate to these circumstances and arrived at a more conservative settling rate of approximately k = 6 m/yr. Walker (1997) countered Qian's criticism by pointing out the visibly superior fit to the WCA-2 data using his parameter estimation technique and by justifying his modeling assumptions.

Researchers at the Duke University Wetland Center (Qian and Richardson 1997; Qian and Reckhow 1998) also developed empirical design models based on the WCA-2 data, but used a different model structure than the first order-models of Walker (1995) and Kadlec and Knight (1996). The Duke models are based on a piece-wise linear relationship between TP loading rate and in-column TP concentration that was derived from review of the North American Data Base (NADB). The piece-wise relationship stated that at loading rates below the breakpoint, phosphorus concentrations are much less sensitive to loading rates than at above the breakpoint. The two Duke teams employed slightly different Bayesian regression techniques, in each case allowing them to refine the collective trends evident in the cross-sectional data set (NADB) with site-specific data from WCA-2. They advocated that this approach (pooling cross-sectional and specific data sets) arrived at a more robust model. The resulting breakpoint from these analyses occurred at a TP loading rate of approximately 1 g m^{-2} yr^{-1} (Qian and Richardson 1997), which they suggested as the maximum "low-risk" mass load to a treatment wetland. A key feature of the Bayesian approach is that it describes prediction uncertainties as the probability of exceeding a given effluent standard, thus allowing users to directly manage the tradeoff between reliability and land area requirements.

Both Duke models predicted more conservatively than did Walker's model. For example, Qian and Reckhow (1998) estimated the total STA land area requirement as 8652 ha, which is approximately three times greater than Walker's estimate (1995). Similarly, Qian and Richardson (1997) estimated Walker's STA design would exceed discharge criteria 75% of the time. During the first four years of operation, the three treatment cells in STA-1W dominated by emergent macrophytes demonstrated an average TP settling rate of k = 11 m/yr with a range of k = 6-17 m/yr (Walker, 1999). Performance during the last two of those four years was quite poor in two of the treatment cells (Cells 1 and 3), averaging k = 3 m/yr. Based on these values, we feel there is insufficient data at this point to judge the relative merits of the empirical models discussed above when they are used in design mode.

Mechanistic Models for P Removal in Wetlands

At the time of this writing, Walker and Kadlec (2000) are in the process of developing a quasi-process model for phosphorus removal in wetlands. The model is called the Dynamic Model for Stormwater Treatment Areas (DMSTA) and is designed specifically for analysis of the District's STAs. We refer to it as a 'quasi-process' model because of its highly aggregated representation of generalized wetland processes (Figure 19). Other process-models in this literature review specify considerably more detail than DMSTA (Figure 18), but this model elaborates significantly beyond the empirical models discussed above. DMSTA was specifically developed as a generalized analysis tool for broad application to emergent vegetation, SAV, and periphyton wetlands.

DMSTA simulates long-term dynamic behavior of phosphorus removal using daily flow and TP concentration input series. The model maintains a daily water balance and can model non-ideal hydraulics using a tanks-in-series (TIS) approach. The model maintains a daily phosphorus mass balance with fluxes (J_{gross}, J_{return}, J_{burial} in Figure 19) between three aggregated storage compartments: water column TP storage (SRP, DOP, PP), short-term labile P storage (biomass, new mineral and organic sediment), and long-term inactive P storage (buried sediment). TP enters the Cell 4 water column in concert with rain and influent flows and also via the recycle

pathway from "labile P storage". TP leaves the water column in concert with effluent flows and with first-order removal to the labile P storage compartment. A fraction of the modeled "active TP storage" is recycled to the water column, accounting for wetland processes such as desiccation, decomposition, dissolution, desorption. The recycle flux is, in essence, a dynamic background (C*) flux. An additional fraction of "active TP storage" is buried to long-term inactive storage; this is the only pathway by which TP is permanently removed from the system. The model predicts a time history of effluent TP concentrations with a daily time step. Each TIS in the DMSTA model can be assigned their own coefficients, thus allowing the model to simulate sequential biological communities.

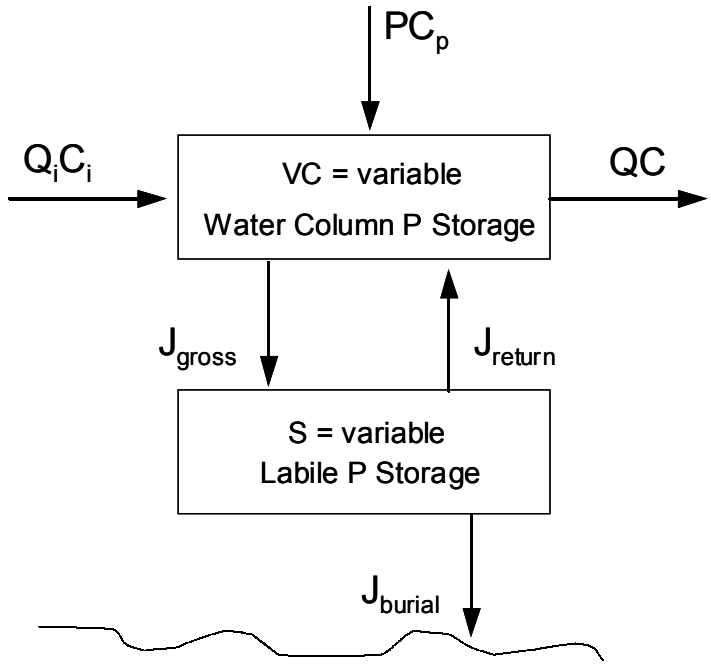


Figure 19. General model structure for DMSTA (Walker and Kadlec 2000) and biomachine models (Kadlec 1997).

In its draft version, DMSTA had three calibration coefficients: the first-order removal constant for TP from the water-column to short-term storage, the fraction of short-term storage that is recycled to the water-column, and the turnover time of the short-term P storage. There is an ongoing research effort to optimize model performance by improving the mathematical relationships defining fluxes between storages (Kadlec 2000); this will likely increase (from 3 to 4 or 5) the number of calibration coefficients.

Kadlec (1997) developed a model with similar structure to DMSTA (Figure 19) but with increased process description. Kadlec's model is called the "Autobiotic Wetland Phosphorus Model" or "biomachine" model. It is based on TP cycling associated with growth, death, and decomposition of biomass (1997). As opposed to the draft DMSTA (Walker and Kadlec 2000), which used a first-order P removal based on water column concentration, the biomachine model ties P removal to the size and turnover rate of the lumped biomass storage. In the biomachine model, the growth and depletion of the labile P storage is described by a logistic growth function (Malthus' Law) with a Monod limiting factor based on the P water concentration. The biomachine model requires calibration of five parameters: maximum total biomass (g/m^2), biomass growth half saturation constant ($g\ P/m^3$), biomass turnover rate (yr^{-1}), TP tissue content in biomass (g P/g), and fraction of senescent biomass (and buried TP) returned to water column. The model predicts a time history of average spatial distributions of TP concentration and biomass within a wetland with a one-year time step (Kadlec 1997). The biomachine model has been successfully calibrated to a 600 ha northern peatland (Kadlec 1997) and to the 26-year WCA-2 data set (Kadlec and Walker 1999).

Mesple et al. (1996) produced one of the only models that we encountered that included both biological and chemical P removal processes (Figure 20). The French researchers developed a mechanistic model for high-rate algal ponds to provide "a rational basis for pond management policies" (Mesple et al. 1996). In their model, biological uptake was modeled as a function of algal growth, death and grazing, where growth rate was a function of temperature, solar radiation, and phosphorus concentration. Their experiments indicated that P precipitation occurred with Ca species when pH elevated due to in-column photosynthesis. In the model, the daily amount of chemically precipitated P was determined using a relationship based solely on weekly measured pH data (i.e. pH was a model input). They justified not including a dependence upon Ca concentration in P precipitation calculations since Ca concentration was relatively constant from week-to-week. This model offered a more detailed representation of water-column processes than the biomachine model, which accounted for only biotic activity (Kadlec 1997). However, the model for the high-rate algal pond did not include any sediment processes, mainly because biomass in a high-rate pond is exported before settling as TSS (they propose that export of particulates, not accrual, is the ultimate removal in a high rate pond).

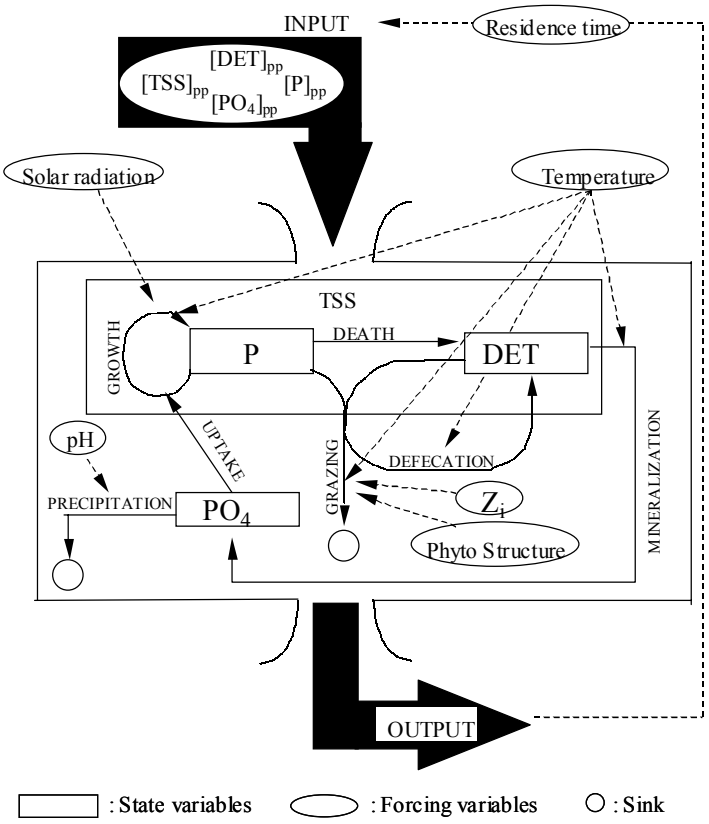


Figure 20. A mechanistic model for high-rate algal ponds that includes both biological uptake and chemical precipitation for P removal (Mesple et al. 1996).

Christensen et al. (1994) produced a mechanistic P removal model that had a moderate amount of water-column and sediment process detail (Figure 21). Their model used six state variables to account for phosphorus storage within the wetland and had approximately 12 calibration coefficients in the equations that defined flow relationships between storages. The six state variables in the model were water volume, dissolved P, particulate P, algal biomass, macrophyte biomass and sediment P. The main goal of the model was to simulate cause-and-effect relationships between system forcing functions (stormwater inflow, pumped inflow, TP concentrations) and ecosystem processes and structure. The time units in the model were weeks, so it was more sensitive to flow and load events than Kadlec's biomachine model (1997), which ran on an annual basis, but less sensitive than the model proposed by Walker and Kadlec (2000), which used a daily time step. The model did not take spatial movements of water or nutrients into account, but it did provide an integrated process description of P removal and

recycling based on the authors' knowledge of this particular wetland (Christiansen et al. 1994). This type of aggregated process model closely follows the systems ecology modeling principles advocated by Odum (1994). Christiansen et al. (1994) cautioned that without an independent data set, it is impossible to validate the model. But despite this shortcoming, they suggest that process models of this type are valuable to field research programs because they can help focus research questions and indicate which parameters should receive the most attention (Christensen et al. 1994).

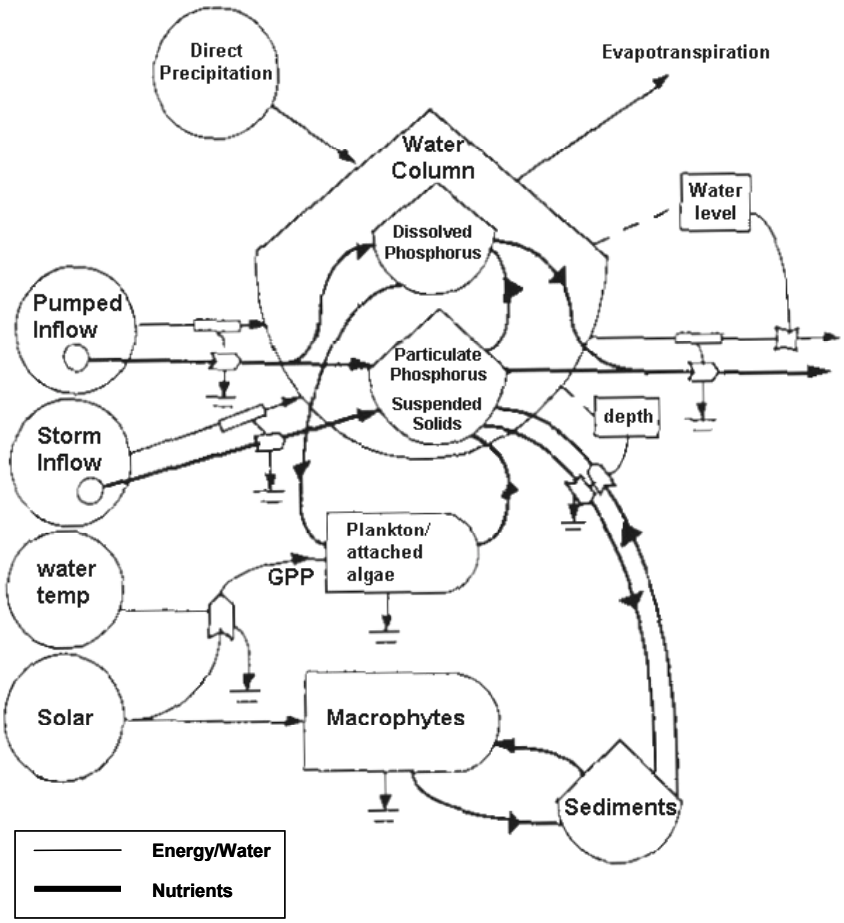


Figure 21. A model of P removal in a northern peatland (Christensen et al. 1994) that captures key process effects based on systems ecology principles.

HydroQual (1997) has developed a multi-dimensional hydrodynamic and water quality model to assist SFWMD water quality managers with the design and operation of STAs. The hydrodynamic component calculates time-variant, two-dimensional water surface elevations and flow patterns. The water quality component computes time-variant, two-dimensional

distributions of dissolved and particulate nutrients. The water quality model consists of four submodels: a periphyton routine, a water chemistry routine, a sediment nutrient flux routine, and an emergent vegetation routine (Figure 22). The modeling equations incorporate over 45 state variables and over 200 calibration coefficients. A sample of some of the state variables tracked by Hydroqual's model includes refractory particulate organic phosphorus, labile dissolved organic nitrogen, solid-phase calcite, sawgrass root carbon, and cattail fallen-dead phosphorus. A sample of some of the model's calibration coefficients include light attenuation factor on saturated periphyton growth rate, reaction velocity for denitrification in the aerobic layer, sawgrass basal respiration rate, first order decay coefficient for accumulated benthic stress, and fraction of root phosphorus lost during root mortality. Spatial resolution, which is a key feature of the model, is provided by dividing a wetland into a grid matrix and solving hydrodynamic and water quality subroutines within each grid cell. For example, the calibration to WCA-2 data utilized a 24x36 matrix (864 cells). Fitz and Sklar (1999) also applied a two-dimensional hydrodynamic and water quality model to WCA-2, using a somewhat different, but equally complex model structure.

DB Environmental's Model Development Objectives and Relationship to Literature Review

The purpose of DBEL's model is to provide a useable tool for performance prediction of SAV treatment wetlands. DBEL's model will serve several roles:

1. Simulation of long-term SAV system performance (forecasting)
2. Simulation of treatment response in SAV systems to highly variable pulse-loadings
3. Simulation of treatment performance of potential SAV systems at other STA locations in which influent water quality and quantity as well as sediment conditions may be variable compared to historical conditions in Cell 4.
4. Research tool to help understand the inter-relationships between key processess identified in subscale experiments.

To provide for these capabilities, DBEL defined the following principles for model development:

1. The model should be an event-driven simulation model and produce a time-history output.

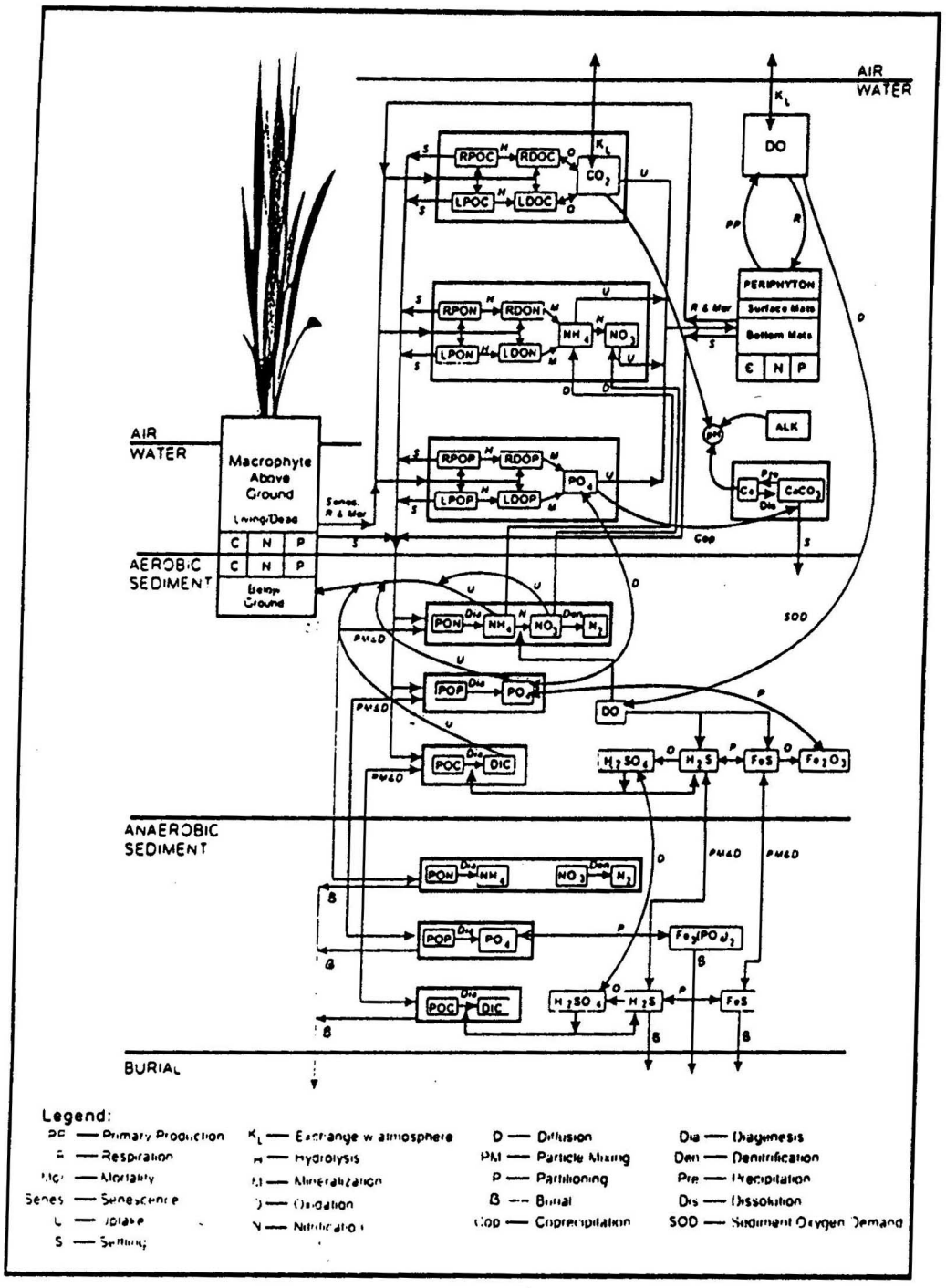


Figure 22. Hydroqual's wetlands water quality model has over 45 state variables and over 200 calibration coefficients.

2. The model should include adequate process detail to capture the influence of key variables that have been identified as significant in microcosm, mesocosm, test cell, and Cell 4 experiments. (i.e., model only phenomena that we have scientifically demonstrated as significant)
3. The model should remain as simple as possible by aggregating all phenomena to the highest possible level without losing variable influence and by leaving out all system variables extraneous to P removal.
4. The model should be developed within the scope of our Task 9 budget.

Based on the above review of scientific literature, we feel that a process model of intermediate complexity, such as those developed by Mesple et al. (1996) and Christensen et al. (1994) would be most appropriate to our goals. Models with less process detail, such as Kadlec and Walker's (2000), under-specify processes to the point where it loses some of the utility we seek as a research tool. On the other hand, complex models such as developed by HydroQual (1997) appear to be overly burdened with detail, making them nearly impossible to calibrate with certainty, and consequently marginally useful for our purposes.

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Task 10. Cell 5 Inoculation and Monitoring

We have sampled STA-1W Cell 5 for water column P concentrations since October 1999 from near the influent structure (G-302). Beginning June 1, 2000, we moved our sampling station to three outfall locations of Cell 5 near the west levee, to monitor the effluent concentrations of TP and apparent color. These preliminary data show a highly colored ($\bar{x}$=310 CPU) effluent with TP concentrations ($\bar{x}$ = 171 µg/L) similar to those found near the influent between October 1999 and May 2000 ($\bar{x}$ = 173 µg/L) (Figure 23).

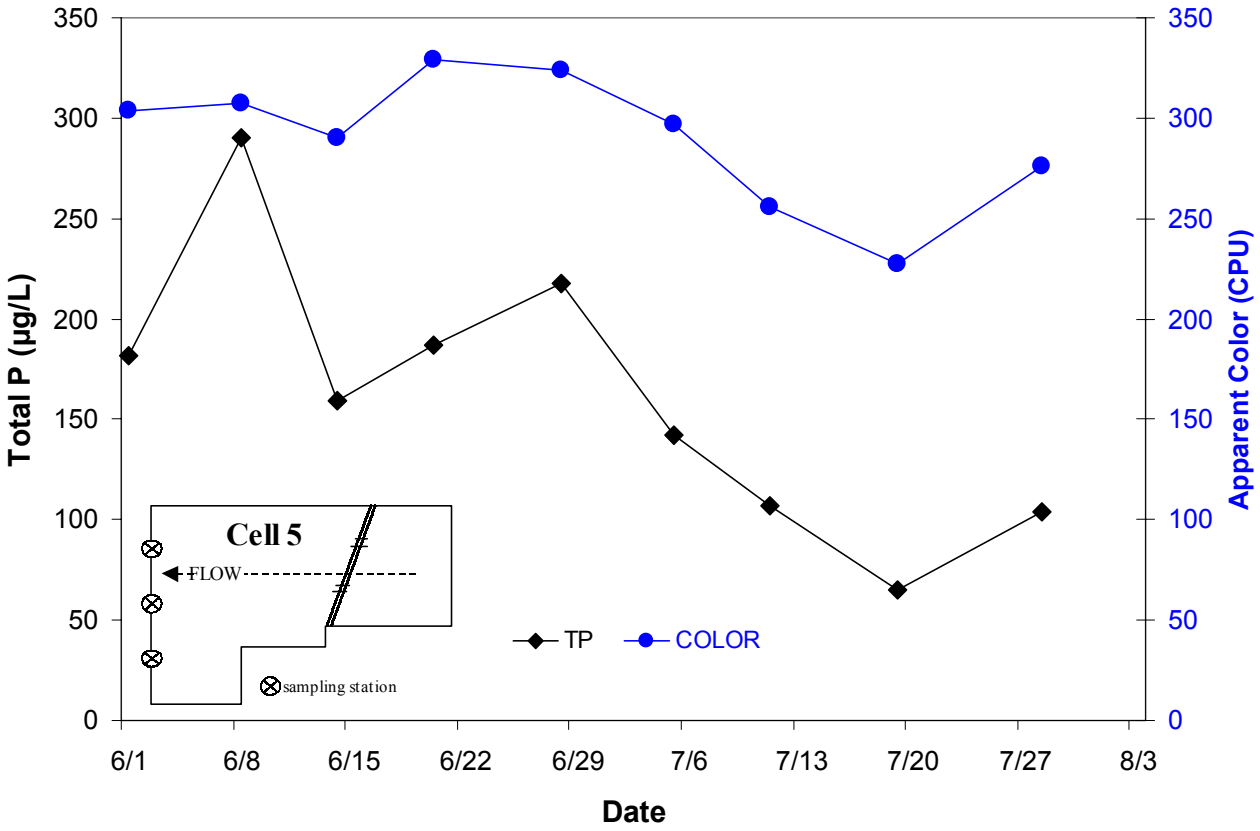

Figure 23. Total phosphorus and apparent color concentrations of surface water near the effluent of Cell 5. Each data period represents the mean of weekly grabs samples from three stations.

Our May 2000 vegetation survey of Cell 5 has revealed that SAV beds, primarily *Najas* and *Ceratophyllum*, have expanded dramatically within the wetland (Figures 24 and 25). SAV is still sparse in the northwest reaches of the cell, however, likely due to the high wave energy created by an unbroken east-west fetch of several km. The low density of SAV in the southwest portion

of the cell is due to the presence of large floating mats of alligator weed (*Alternanthera*). At this rate of colonization, the vegetation should soon reach sufficient density to improve TP removal throughout the cell.

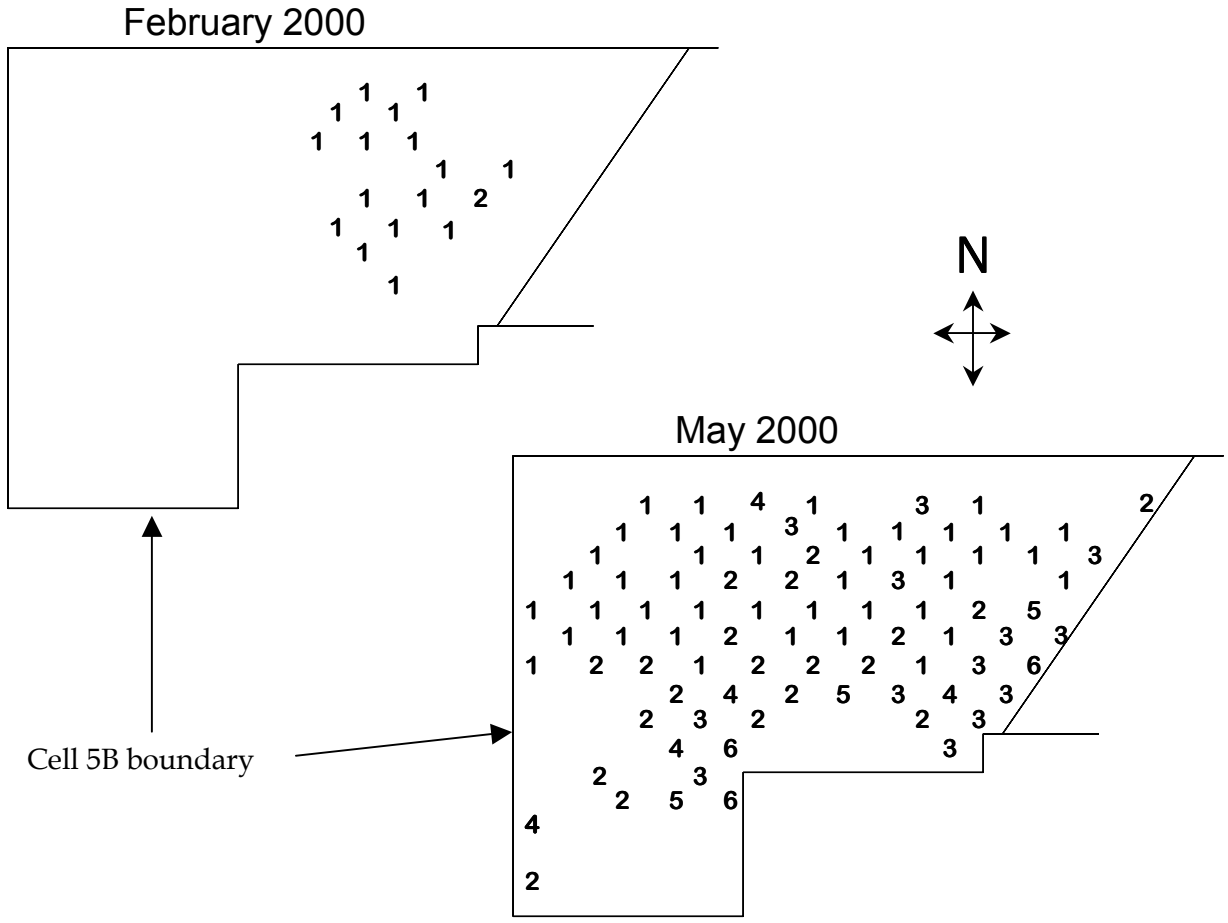

Figure 24. Distribution of *Najas guadalupensis* throughout STA-1W Cell 5 during February and May 2000, based on qualitative sampling at 120 evenly-spaced locations. Numbers reflect relative density from very sparse (1) to dense (6).

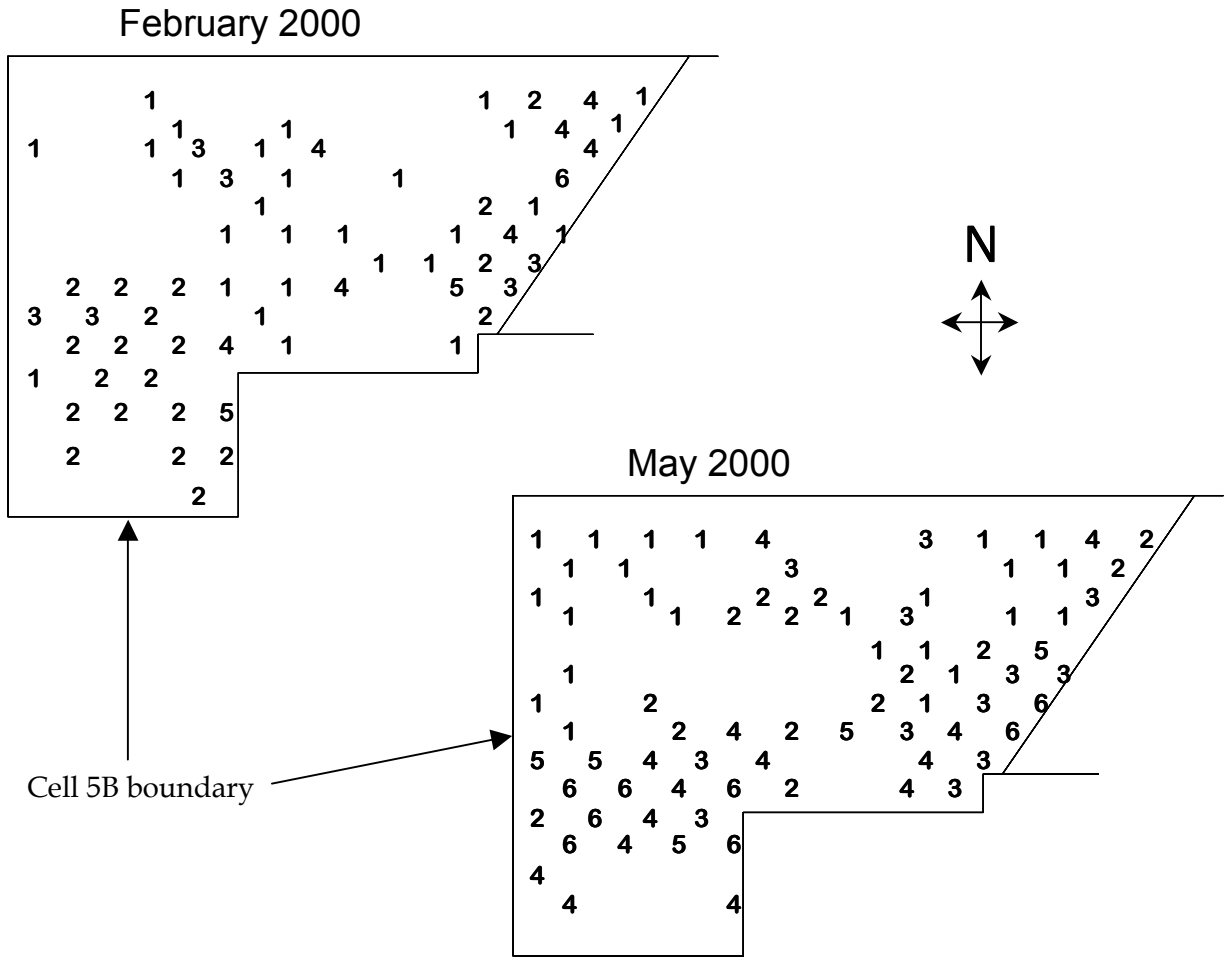

Figure 25. Distribution of *Ceratophyllum demersum* throughout STA-1W Cell 5 during February and May 2000, based on qualitative sampling at 120 evenly-spaced locations. Numbers reflect relative density from very sparse (1) to dense (6).